

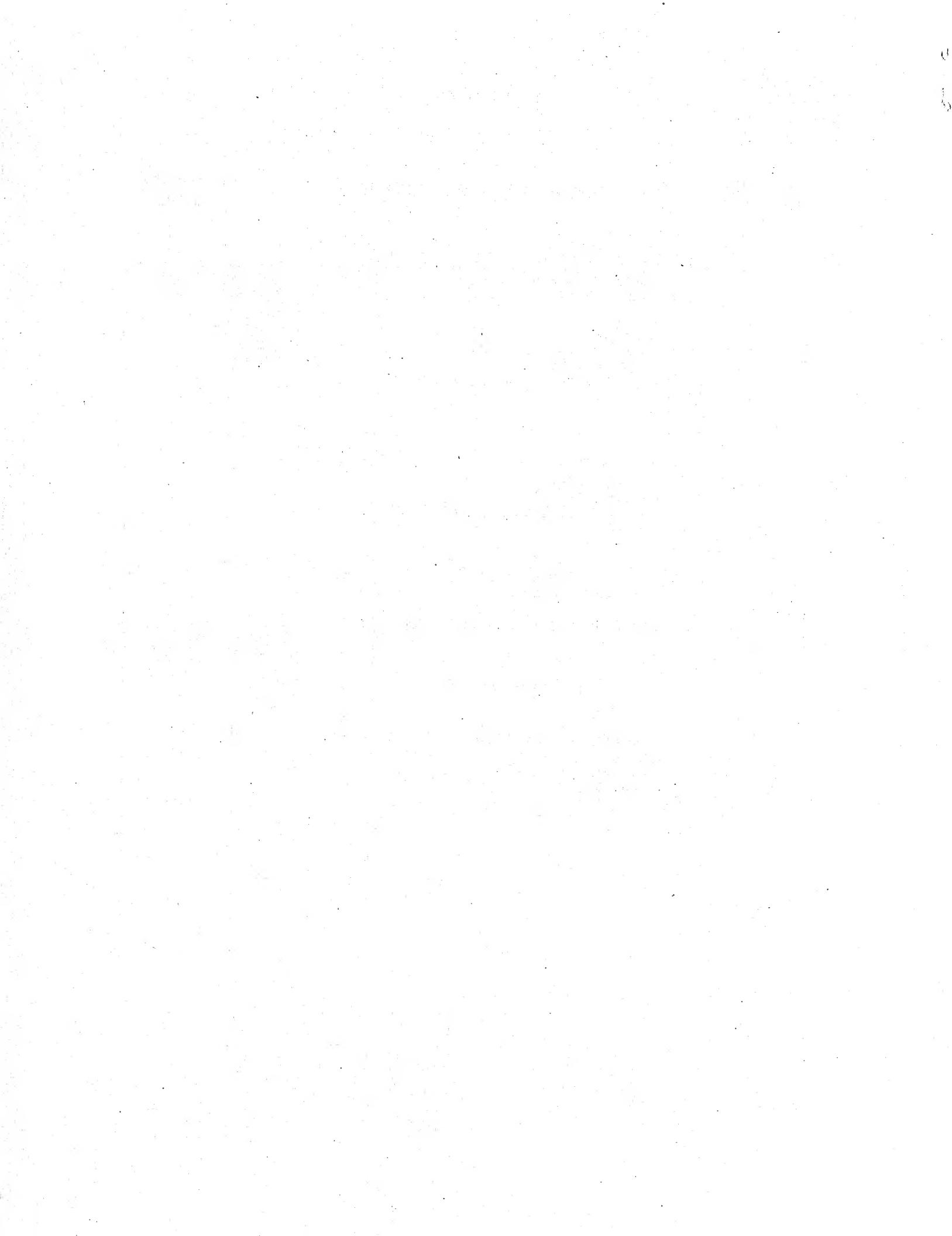


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EUROPEAN SEARCH REPORT

Application Number
EP 94 30 5409

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
D, Y	DATABASE WPI Section PQ, Week 9201, Derwent Publications Ltd., London, GB; Class P83, AN 92-004699 & JP-A-3 259 242 (KONICA CORPORATION) 19 November 1991 * abstract * ---	1,6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
Y	DATABASE WPI Section PQ, Week 8216, Derwent Publications Ltd., London, GB; Class P83, AN 82-32025E & JP-A-57 044 140 (MITSUBISHI PAPER MILLS LTD) 12 March 1982 * abstract * ---	1,6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
Y	US-A-4 618 573 (H.OKAMURA ET AL) *column 21,compounds H-33,H-34* ---	1,6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
Y	GB-A-1 076 629 (GEVAERT PHOTO-PRODUCTEN NV) * page 2, line 1 - line 10 * ---	1,6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
A	US-A-3 428 456 (H GRABHÖFER ET AL) * the whole document * ---	6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
A	EP-A-0 524 540 (FUJI PHOTO FILM CO. LTD.) * page 5, line 29 - line 45 * *page 35, structure a-VI-2* * page 36, line 17 - line 23 * *page 61,compound E-30* -----	6,11	G03C1/30 C09H7/00 G03F7/07 //C07F9/09
The present search report has been drawn up for all claims			G03C1/30 C09H7/00 G03F7/07 //C07F9/09
Place of search		Date of compilation of the search	Examiner
THE HAGUE		24 November 1994	Bolger, W
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	



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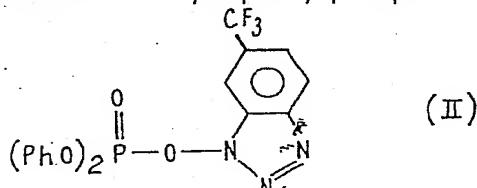
C2P 1L2 3B13 3B15A 3B18B
C2C 1314 1382 1440 1464 1470 1492 1530 1544 214
215 246 247 250 251 253 256 25Y 28X 290 29X 29Y
30Y 321 32Y 33Y 340 342 346 34Y 351 352 366 367
368 373 37Y 614 628 638 670 801 80Y DE KE RP

(56) Documents cited
None

(58) Field of search
C2P

(54) 6-trifluoromethyl-1H-benzotriazol-1-yl diphenylphosphate and its use in preparing cephalosporin derivatives

(57) The invention relates to the preparation of cephalosporin derivatives using 6-trifluoromethyl-1H-benzotriazol-1-yl diphenyl phosphate of formula II



as a condensing agent, to a novel condensing agent of formula II and to process for the preparation of that condensing agent.

GB 2 193 213 A

Formulae in the printed specification were reproduced from drawings submitted after the date of filing, in accordance with Rule 20(14) of the Patents Rules 1982.

This print takes account of replacement documents submitted after the date of filing to enable the application to comply with the formal requirements of the Patents Rules 1982.

SPECIFICATION

6-Trifluoromethyl-1H-benzotriazole-1-yl diphenylphosphate and its use in preparing cephalosporin derivatives

5 BACKGROUND OF THE INVENTION

This invention relates to a process for preparing cephalosporin derivatives.

It is well known that the acylation process is carried out by using acid chloride or mixed acid anhydride, and also the process using reactive acid derivatives converted from organic acid with 10 1-hydroxysuccinimide or 1-hydroxy-1H-benzotriazole has been developed. These processes have merits and demerits, but the acylation using 1-hydroxy-1H-benzotriazole is particularly favoured.

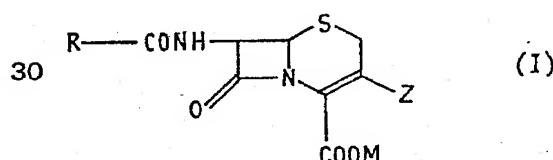
However, the acylation using 1-hydroxy-1H-benzotriazole has disadvantage of taking long reaction time. For example the acylation processes disclosed by West German Patent No. 3316798 and British patent No. 2098216 using the reactive derivatives prepared from 1-hydroxy-1H-benzotriazole by the method of dicyclo carbodiimide require much reaction time. The reactive derivatives prepared for 3~4 hours have to be reacted for one night at room temperature to complete the acylation.

BRIEF SUMMARY OF THE INVENTION

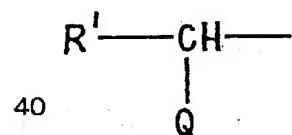
20 The object of the present invention is to provide a continuous process for the preparation of cephalosporin derivatives with high yield and purity in short reaction time, without carrying out separately the processes for the preparation of condensate, reactive derivatives, and acylation which is the disadvantage of the prior art.

The present invention will be more clearly explained in the following.

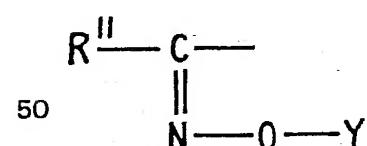
25 According to the present invention, there is provided a novel method for preparing cephalosporin derivatives of compound of the general formula (I).



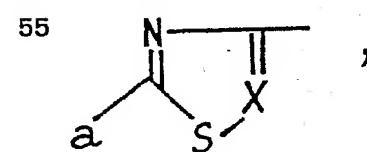
35 wherein R is α -substituted arylmethyl of the general formula



(R' is phenyl or phenyl substituted by hydroxy or halogen, Q is protective hydroxy, protective amino or protective carboxy, or substitution group of the form of ureido), or -oxyimino group of 45 the general formula



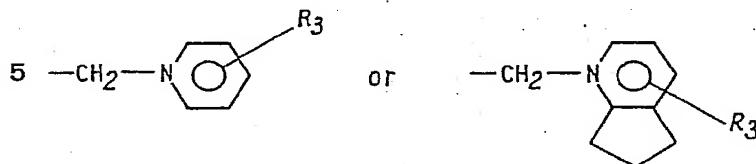
(where R'' is furyl, or



60 where a is H or NH₂, X is CH or N, and Y is hydrogen, C₁—C₅ alkyl, alkenyl, alkynyl, or cycloalkyl group),

Z is hydrogen, halogen atom or an alkyl, cycloalkyl, alkoxy or alkylthio groups containing less than five carbon atoms, or acetoxy methyl or carbamoyloxy methyl group, or —CH₂—S—R₂ or 65 —CH₂—R₂, where R₂ is heterocycle of five to six ring members containing one to four heteroatom

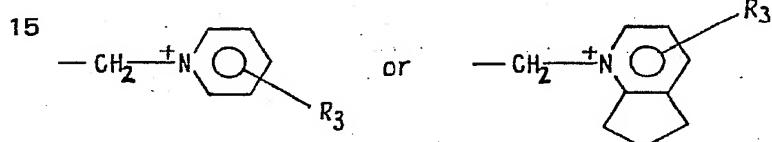
arbitrarily substituted from S and N, or



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10 where R₃ is hydrogen, alkyl containing one to five carbon atoms, ester, amide, or organic acid group, M is hydrogen, alkali or alkaline earth metal, or ester group, or COOM group represents CO₂, and also COO⁻ when Z represents

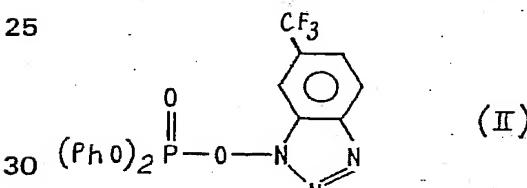
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20 The method of the present invention is characterised in that the reactive derivatives obtained from compound of the general formula (III) by treating with condensing agent of the general formula (II) in the presence of organic base is reacted with compound of the general formula (IV)

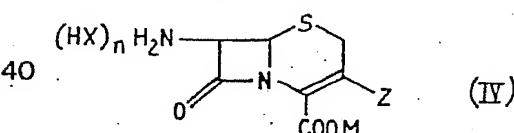
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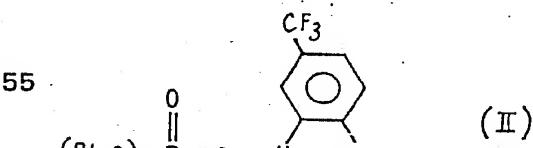
40 where R, Z and M are the same as represented hereinbefore, X is a halogen atom, and n is an integer of zero to two.

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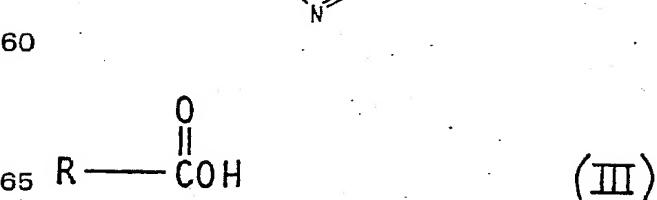
DETAILED DESCRIPTION

45 According to the present invention, syn-isomer of compound of the general formula (I) described hereinbefore is prepared from reacting the reactive derivatives, which is obtained from 50 novel condensing agent of the compound of the general formula (II) reacting with organic acid of the general formula (III), with compound of the general formula (IV).

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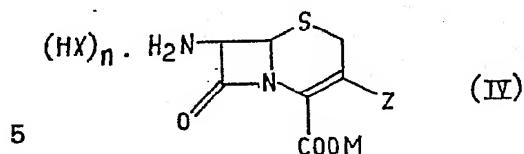


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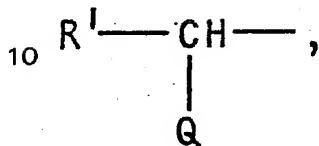
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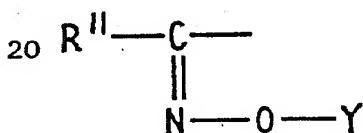
wherein R is α -substituted arylmethyl or heteroaryl methyl of the general formula



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15 (where R' is phenyl, phenyl substituted with halogen or hydroxy, Q is protective hydroxy, protective amino or protective carboxy, or substituted group of the form of ureido), or α -oxyimino group of the general formula

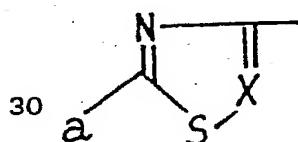
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25 (where R'' is furyl or

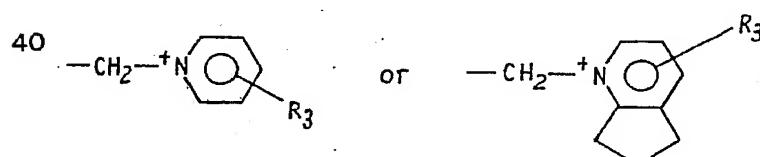
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35 in which a is hydrogen or amino group, Z is CH or N, Y is hydrogen, C₁—C₅ alkyl, alkenyl, alkynyl or cycloalkyl, Z is hydrogen or halogen atom, an alkyl, cycloalkyl, alkoxy or alkylthio groups containing less than five carbon atom, or acetoxy methyl or carbamoyloxymethyl, or —CH₂—S—R₂ or —CH₂—R₂ in which R₂ is heterocycle of five to six ring members containing one to four heteroatom arbitrarily substituted from S and N, or

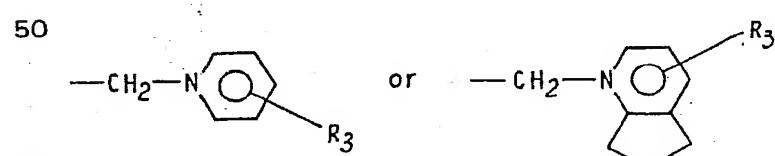
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45 in which R₃ is hydrogen, alkyl containing one to five carbon atoms, ester, organic acid or amide group, M is hydrogen atom, alkali, alkaline earth metal, or ester group, or COOM group represents CO₂, and also COO⁻ when Z represents

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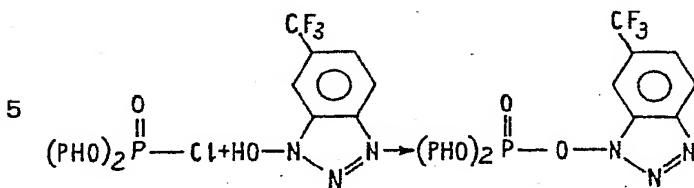


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55 X is a halogen atom, and n is an integer of zero to two.
Hereinafter, the present invention will be described more specifically.
6-Trifluoromethyl-1H-benzotriazole-1-yl-diphenyl phosphate of compound of the general formula (II) using as a novel condensing agent in the process of the present invention may be quantitatively prepared and isolated by using solvent of tetrahydrofuran, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, or N-methylpyrrolidone (Example 1), but it is preferable to react it directly with an organic acid without separation.

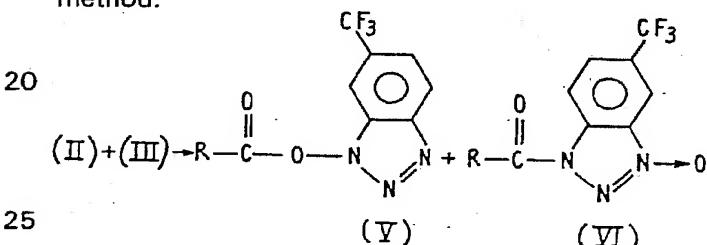
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10 Hydrochloric acid formed during the reaction is neutralised by using triethylamine. 10

The organic acid of the general formula (III) is dissolved in solvent of N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone, and then reacted with the condensing agent of the general formula (II) at $0 \pm 5^\circ\text{C}$ by using trimethylamine or pyridine as base; the reaction is carried out quantitatively within a few minutes. The reactive derivative formed hereinabove is 15 possible to confirm by separation in Examples as the mixtures of the general formulae (V) and (VI), but the present invention carries out acylation without separation as in the conventional method.



Carrying out the acylation by addition of 7-amino cephalosporin derivative of the general formula (IV) dissolved in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone to the reactive derivative formed during the reaction, the cephalosporin derivative of the general 30 formula (I) that is the object of the present invention can be prepared with high yield.

The temperature of acylation is preferred to be between 0°C and room temperature.

As described hereinbefore, the present invention is characterised in that acylation is carried out by using the compound of the general formula (II) of condensing agent, especially has the advantage of preparing continuously in one reaction vessel without separately carrying out the 35 process of preparations of condensing agent, reactive derivatives, and acylation.

According to the method of the present invention, there is obtained a cephalosporin derivative with various antibiotic functions, and besides the products mentioned in the Examples, the present invention especially provides the following compounds:

7-[(2-amino-4-thiazolyl)-2-methoxyimino acetyl]amino]ceph-3-em-4-carboxylic acid, syn-isomer 40 alkali or alkali metal, ammonium salt, consisting of amino organic base and ester with degradable group.

7-[(2-amino-4-thiazolyl)-2-methoxyimino acetyl]-3-(1-methyl-1-pyrrolidinium) methyl]-3-cephem-4-carboxylate, syn-isomer.

7-[(2-fur-2-yl)2-methoxyimino acetyl]amino]-3-carbamoyloxymethyl ceph-3-em-4-carboxylic acid, 45 syn-isomer thereof salt thereof and ester with degradable group.

7-[(2-thiazolyl)-2-(carboxymethoxyimino) acetyl]amino]ceph-3-em-4-carboxylic acid.

N-[7-[(2-(5-amino-1,2,4-thiadiazol-3-yl) 2-ethoxyamino acetyl]amino]3-cephem-3-yl]methyl]-pyridinium-4-carboxylate, syn-isomer and salt thereof.

The present invention will be described in more detail by the typical Examples hereinafter.

50 Example 1

To the solution of 2.30 g (10 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 15 ml of tetrahydrofuran, 2.07 ml (10 millimoles) of diphenyl chloro phosphate is added dropwise under ice-bath condition, and then 1.39 ml of triethylamine (10 millimoles) is 55 slowly added at the same temperature. After stirring for 2 hours at room temperature, the solid formed during the reaction is filtered off, and crystallised with petroleum ether. Filtering and drying the crystalline, white 6-trifluoromethyl-1H-benzotriazole-1-yl-diphenyl phosphate is obtained Yield: 4.17 g (96%).

Melting Point: 112~115 $^\circ\text{C}$

60 IR_{max}, KBr, cm^{-1} : 1315 ($\text{P}=\text{O}$), 1245 ($\text{P}-\text{O}-\text{N}$)

NMR (DMSO- d_6 , δ): 7.10~7.45 (10H, m)

7.60~7.80 (1H, m)

8.15~8.35 (2H, m)

To the solution of 4.0 g (20 millimoles) of 2-(2-amino-4-thiazolyl)-2-syn-methoxyimino acetic acid dissolved in 40 ml of N,N-dimethylformamide, 3.1 ml (22 millimoles) of trimethylamine is added dropwise at 5°C and 9.75 g (22 millimoles) of 6-trifluoromethyl-1H-benzotriazole-1-yl-diphenyl phosphate is added at the same temperature, and then the reaction mixture is stirred for 30 minutes. Filtering and drying the crystalline under reduced pressure formed by adding the reactant into ice water, white mixture or syn-(2-amino-4-thiazolyl)-(methoxyimino) acetic acid-6-trifluoromethyl-1H-benzotriazole-1-yl ester and syn-1-[(2-amino-4-thiazolyl)-(methoxy imino) acetyl]-3-hydroxy-5-trifluoromethyl-1H-benzotriazolium, hydroxide inner salt. Yield: 6.9 g (90%)

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100

10 Melting point: 198°C (decomposition)
IR (λ_{max} , KBr, Cm^{-1}):
NMR (DMSO-d₆, δ): 3.90 (3H, S), 6.84 (1H, S)
7.56~8.25 (3H, m)

15 Example 3
To the solution of 0.96 g (2 millimoles) of 7-amino-3-[(2.3-cyclopenteno-1-pyridinio)methyl]-3-cephem-4-carboxylate iodic acid hydride dissolved in 10 ml of N,N-dimethylformamide, 0.95 g (2.1 millimoles) of the mixture of syn-(2-amino-4-thiazolyl)-(methoxyimino) acetic acid 6-trifluoromethyl-1H-benzotriazole-1-yl ester and syn-1-[(2-amino-4-thiazolyl)-(methoxyimino)acetyl]-3-hydroxy-5-trifluoromethyl-1H-benzotriazolium hydroxide inner salt is added under freezing condition, and then stirred for 1 hour at room temperature. After completing the reaction, insoluble materials are filtered off. After removing solvent by the distillation under reduced pressure (2 mmHg), 10 ml of isopropylalcohol is added to the residue to crystallise, and then the mixture is stirred for 30 minutes under freezing condition. Filtering and drying under reduced pressure, light yellowish 7-[[2-(2-amino-4-thiazolyl)-2-syn-methoxyimino]acetamido]-3-(2.3-cyclopenteno)-3-cephem-4-carboxylate iodic acid is obtained.
Yield: 1.0 g (83.3%)
Melting Point: 179~181°C (decomposition)
IR (λ_{max} , KBr, Cm^{-1}): 1785 (lactum C=O)
NMR (CF₃COOD, δ): 2.30~2.85 (2H, m)
3.10~4.05 (6H, m)
4.41 (3H, S) 5.21~6.23 (4H, m)
8.11 (1H, S) 7.65~8.70 (3H, m)

20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100

35 Example 4
To the solution of 2.96 g (6.8 millimoles) of 6-trifluoro methyl-1H-benzotriazole-1-yl-diphenyl-phosphate dissolved in 90 ml of N,N-dimethylformamide, the solution of 1.2 g (6 millimoles) of 2-(2-amino-4-thiazolyl)-2-syn-methoximino acetic acid dissolved in 12 ml of N,N-dimethylformamide is slowly added under freezing condition, and 0.92 ml (6.6 millimoles) of triethylamine is slowly added at the same temperature. After stirring 30 minutes under freezing condition, 2.6 g (5.4 millimoles) of 7-amino-3-[(2.3-cyclopentano-1-pyridinio)methyl]-3-cephem-4-carboxylate iodic acid hydride is added, and the reactant is stirred for 1 hour at room temperature. Treating the reactant with the same procedure described in Example 3, light yellowish 7-[(2-(2-amino-4-thiazolyl)-2-syn-methoxyimino)acetamino]-3-(2.3-cyclopenteno)-3-cephem-4-carboxylate iodic acid is obtained [Yield: 40 g (94%)].
1R and NMR data are the same as Example 3.

40 45 50 55 60 65 70 75 80 85 90 95 100

Example 5
To the solution of 2.03 g (10 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 20 ml of N,N-dimethylformamide, 2.07 ml (10 millimoles) of diphenylchlorophosphate is added dropwise under freezing condition, and 1.39 ml (10 millimoles) of triethyl amine is slowly added at the same temperature. After stirring for 1 hour at room temperature, the solution of 2.0 g (10 millimoles) of 2-(2-amino-4-thiazolyl)-2-syn-methoxy imino acetic acid dissolved in 20 ml of N,N-dimethylformamide is slowly added to the solution hereinbefore, and 1.39 ml (10 millimoles) of triethylamine is added. After stirring for 30 minutes at room temperature, solution of 4.8 g (10 millimoles) of 7-amino-3-[(2.3-cyclopenteno-1-pyridinio)methyl]-3-cephem-4-carboxylate iodic acid hydride dissolved in 40 ml of N,N- dimethylformamide is added, and then stirred for 1 hour at room temperature.
Treating the reactant with the same procedure described in Example 3, light yellowish 7-[(2-(2-amino-4-thiazolyl)-2-syn-ethoxy amino)acetamido]-3-(2.3-cyclopentano)-3-cephem-carboxylate iodic acid is obtained. The yield is 7.4 g (94%), IR and NMR data are the same as Example 3.

60 65 70 75 80 85 90 95 100

Example 6
To the solution of 1.02 g (5 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 10 ml of N,N-dimethylformamide, 1.03 ml (5 millimoles) of diphenylchlorophosphate is

added dropwise under freezing condition, and 0.7 ml (5 millimoles) of triethylamine is slowly added dropwise at the same temperature. After stirring 1 hour at room temperature, the solution to 1.37 g (5 millimoles) of 2-(2-amino-4-thiazolyl)-2-syn-(2-butoxy carbonylpropyl-2-oxyamino) acetic acid dissolved in 20 ml of N,N-dimethyl formamide is slowly added to the solution

5 hereinbefore, and 0.7 ml (5 millimoles) of triethylamine is added. After stirring for 30 minutes at room temperature, solution of 1.82 g (5 millimoles) of 7-amino-3-(1-pyridinium) methyl-3-cephem-4-carboxylate bis hydrochloric acid salt dissolved in 10 ml of N,N-dimethylformamide is slowly added dropwise, and then reacted for 1 hour at room temperature. After removing solvent by the distillation under reduced pressure, 20 ml of 98% formic acid is added to the

10 residue, and stirred for 40 minutes at room temperature. After distillating the filtrate under reduced pressure, 30 ml of acetone is added to the residue of organic phase to crystallise. Filtering and drying the crystalline under reduced pressure, 7-[2-(2-amino-4-thiazolyl)-2-syn-(2-carboxyprop-2-oxyamino) acet amido]-3-(pyridinium methyl)-3-cephem-4-carboxylate bis salt is obtained

15 Yield: 2.5 g (85%)

NMR ($D_2O/NaHCO_3$, δ): 1.45 (6H,S) 6.26 (1H,d,J=50Hz)
5.84 (1H,d,J=5Hz) 6.90 (1H,S)
8.05~9.05 (5H,m)

20 Example 7

To the solution of 2.03 g (10 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 20 ml of N,N-dimethylformamide, 2.07 ml (10 millimoles) of diphenylchlorophosphate is added dropwise under freezing condition, and 1.39 ml (10 millimoles) of triethylamino is slowly added dropwise at the same temperature. After stirring for 1 hour at room temperature, the

25 solution of 4.1 g (10 millimoles) 7-amino-3-[2-(5-methyl-1,2,3,4-tetrazolyl)methyl]-3-cephem-4-(pyvaloyl oxymethyl) carboxylate hydrochloric acid salt dissolved in 20 ml of N,N-dimethylformamide is slowly added dropwise, and reacted for 1 hour at room temperature. After adding the reactant said above to the mixture of water and ethyl acetate, PH is adjusted to 7.0 under freezing condition, and organic phase is separated. After removing solvent by the distillation

30 under reduced pressure, the solid is produced by adding ethylether to the residue. Filtering and drying under reduced pressure, 7-[2-(2-amino-4-thiazolyl)-2-syn-methoxyamino acetamido]-3-[2-(5-methyl-1,2,3,4-tetrazolyl)methyl]-3-cephem-4-(pyvaloyloxy methyl) carboxylate is obtained

Yield: 5.3 g (90%)

Melting Point 127~128°C (decomposition)

35 IR (λ_{max} , KBr, Cm^{-1}): 1780, 1743, 1765

Example 8

To the solution of 2.03 g (10 millimoles) of 1-hydroxy-6-trifluoroethyl-1H-benzotriazole dissolved in 20 ml of N,N-dimethylformamide, 20 ml (10 millimoles) of diphenylchlorophosphate is added dropwise under freezing condition, and 1.39 ml (10 millimoles) of triethylamine is slowly added dropwise at the same temperature. After stirring for 1 hour at room temperature, the solution of 3.32 g (10 millimoles) of 7-amino-3-[2-(5-methyl-1,2,3,4-tetrazolyl)methyl]-3-cephem-4-carboxylate hydrochloride dissolved in 20 ml of N,N-dimethylformamide is slowly added dropwise, and reacted for 1 hour at room temperature.

45 After removing solvent by distillation under reduced pressure, 10 ml of isopropyl alcohol is added to the residue to crystallise, and then stirred under freezing condition for 30 minutes. Filtering and drying under reduced pressure, there is obtained light yellowish 7-[2-(2-amino-4-thiazolyl)-2-syn-methoxyimino-acetamido-3-2-(5-methyl-1,2,3,4-tetrazolyl)methyl]-3-cephem-4-carboxylate.

50 Yield: 4.3 g (90%)

Melting Point: 184~187°C (decomposed)

IR (λ_{max} , KBr, Cm^{-1}): 1765, 1655, 1630

NMR ($CF_3 COOD$, δ): 2.43 (3H, S), 3.45 (4H, bs),
5.08 (1H, d), 5.50 5.90 (3H, m),
6.37 (1H,S)

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Example 9

To the solution of 1.09 g (5.4 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 20 ml of N,N-dimethylformamide, 1.1 ml (5.4 millimoles) of diphenyl chlorophosphate is added dropwise under freezing condition, and 0.75 ml (5.4 millimoles) of triethylamino is slowly added at the same temperature. After stirring at room temperature for 1 hour, 1.97 g (5.4 millimoles) of 7-amino-3-(1-methyl-1-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate hydrochloride salt is added, and stirred for 30 minutes at room temperature. After completing the reaction, the reactant is poured into a water and the PH of the solution is adjusted to 2.0 with 2N-hydrochloric acid, and then stirred at room temperature for 1 hour. After freezing, 7-[D(-)- α -

60

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(4-hydroxy-6-methylpyridine-3-carboxamido)- α -hydroxyphenyl)acetamide]-3-[(1-methyl-1H-tetrazole-5-yl)thiomethyl]-3-cephem-4-carboxylic acid is obtained by filtration.

Yield: 3.10 g (94%)

Melting Point: 204~206°C (decomposed)

5 IR: (λ max, KBr, Cm^{-1}): 1770, 1660, 1635

NMR (DMSO-d₆, δ): 2.3 (3H,S), 3.6 (2H,g), 3.9 (3H,S)
4.3 (2H,g), 5.1 (1H,d), 5.5~5.8,
(2H,m), 6.3(1H,S), 6.7~7.5 (4H,g)
8.3 (1H,m)

10

5

Example 10

To the solution of 1.09 g (5.4 millimoles) of 1-hydroxy-6-trifluoromethyl-1H-benzotriazole dissolved in 20 ml of N,N-dimethylformamide, 1.1 ml (5.4 millimoles) of diphenyl phosphate is added dropwise, and 0.75 ml (5.4 millimoles) of triethylamino is slowly added. After stirring at

15 room temperature for 1 hour, 2.46 g (5.4 millimoles) of (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl-7-amino-3-[(5-methyl-1,3,4-thiadiazole-2-yl)thio]methyl]-3-cephem-4-carboxylate is added, and 15

stirred for 1 hour at room temperature. After removing solvent under reduced pressure (2 mmHg), methanol is added to the residue to crystallise. Adding concentrated hydrochloric acid to the solution of crystalline dissolved in acetone at 5°C, stirred and filtered at room temperature,

20 (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl-7-[D-O-(L-alanyl)mandelamido]-3-[(5-methyl-1,3,4-thiadiazole-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride salt is obtained.

Yield: 6.3 g (90%)

Melting Point: 158°C (decomposed)

1R (Nujol, Cm^{-1}): 1820, 1780, 1740, 1690

25 NMR (DMSO-d₆, δ): 1.48 (d, 3H), 2.18 (S, 3H), 2.66, (S, 3H, 3.62 (S, 2H), 4.2 (1H, m), 4.07, 4.65 (g, 2H), 5.05 (d, 1H), 5.15 (S, 2H), 5.72 (d of d, 1H), 6.13 (S, 1H), 7.4 (m, 5H), 8.73 (bs, 3H), 9.46 (d, 1H)

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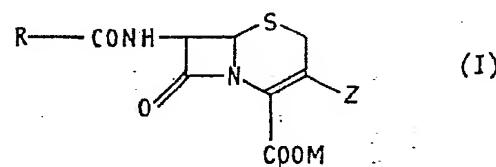
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CLAIMS

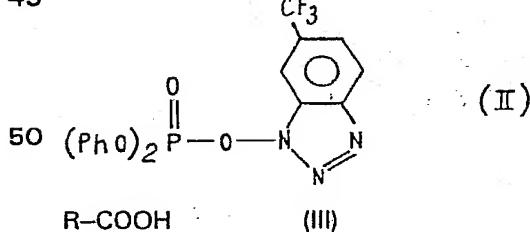
1. A process for the preparation of cephalosporin derivatives of the general formula (I), characterised by reacting organic acid of the following general formula (III) with compound of the 35 following general formula (IV) under the presence of condensing agent of the following general formula (II)

35



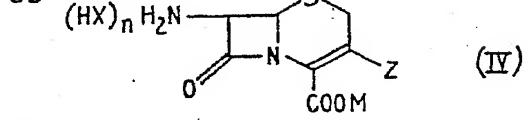
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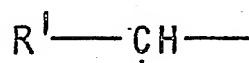


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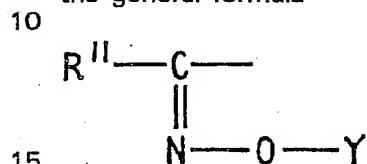


60 wherein R is α -substituted arylmethyl of the general formula



5

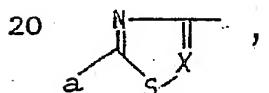
(R' is phenyl or phenyl substituted by hydroxy or halogen, Q is protective hydroxy, protective amino or protective carboxy, or substitution group of the form of ureido) or α -oxyimino group of the general formula



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(where R'' is furyl, or



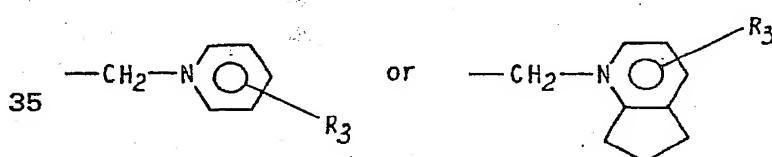
20

25 where a is H or NH₂, X is CH or N, and Y is hydrogen, C₁—C₅ alkyl, alkenyl, alkynyl or cycloalkyl group),

25

Z is hydrogen, halogen atom or an alkyl, cycloalkyl, alkoxy, or alkylthio groups containing less than five carbon atoms, or acetoxyethyl or carbamoyloxyethyl group, or —CH₂—S—R₂ or —CH₂—R₂, where R₂ is heterocycle of five to six ring members containing one to four heteroatom arbitrarily substituted from S and N, or

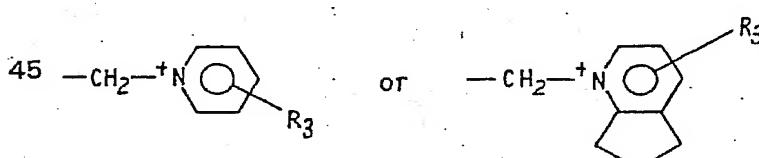
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40 where R₃ is hydrogen, alkyl containing one to five carbon atoms, ester, amide, or organic acid group, M is hydrogen, alkali or alkaline earth metal, or ester group, or COOM group represents CO₂, and also COO when Z represents

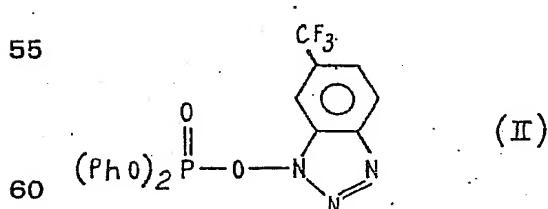
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50 X is a halogen atom, and n is an integer of zero to two.
2. A condensing agent of the formula (II) in Claim 1.

50



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60 3. A process for the preparation of cephalosporin derivatives substantially as described herein with reference to Examples 1 to 10.

60

65 4. A condensing agent substantially as described herein with reference to Examples 1 to 10.

65

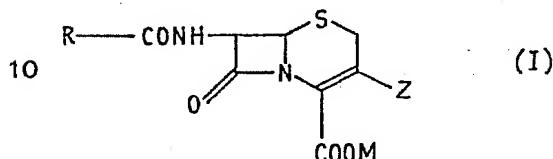
CLAIMS

Amendments to the claims have been filed, and have the following effect:—

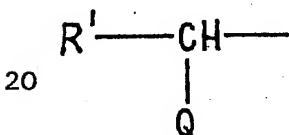
Claims 1 to 4 above have been deleted or textually amended.

New or textually amended claims have been filed as follows:—

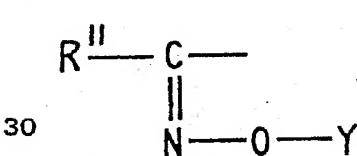
5 1. The compound which is 6-trifluoromethyl-1H-benzotriazol-1-yl-diphenyl phosphate. 5
2. A process for preparing a cephalosporin derivative of the formula



15 wherein R is α -substituted arylmethyl of the formula 15



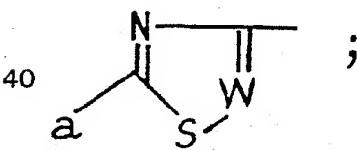
25 or α -oxyimino of the formula 25



35 R' is optionally-substituted aryl (homocyclic or heterocyclic); 35

Q is protected hydroxy, protected amino, protected carboxy or a substitution group in the form 35 of ureido; 35

R'' is furyl or



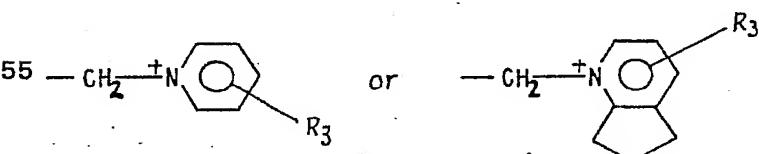
45 a is a hydrogen atom (—H) or $—\text{NH}_2$;

W is $=\text{CH}-$ or $=\text{N}-$;

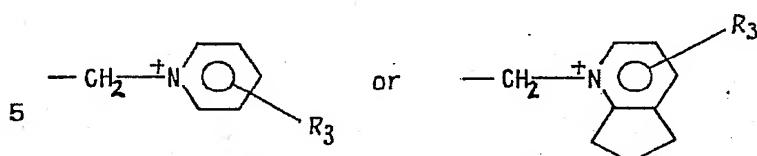
Y is a hydrogen atom (—H), C_{1-5} -alkyl, C_{2-5} -alkenyl, C_{2-5} -alkynyl or cycloalkyl;

Z is a hydrogen atom (—H), halo, C_{1-4} -alkyl, C_{1-4} -alkoxy, C_{1-4} alkylthio, cycloalkyl having at most six ring carbon atoms, acetoxyethyl, carbamoyloxy-methyl, $—\text{CH}_2—\text{S—R}_2$ or $\text{CH}_2—\text{R}_2$;

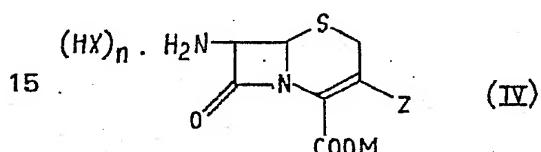
50 R_2 is an optionally-substituted heterocycle of five or six ring members and having from one to four ring hetero atoms, each of which is S or N, 50



60 R_3 is a hydrogen atom (—H), C_{1-5} -alkyl, an ester, amide or organic acid group; and 60
M is a hydrogen atom (—H), an alkali metal, an alkaline-earth metal or an ester group; or COOM represents CO_2 ; or, when Z is

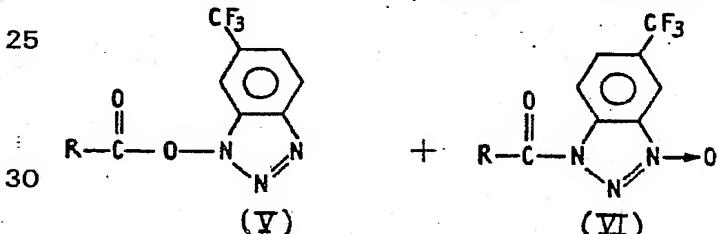


COOM is optionally COO-;
 10 which comprises reacting organic acid of the formula R-COOH
 wherein R has its previously-ascribed meaning, with a compound of the formula

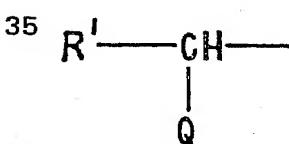


wherein M and Z have their previously-ascribed meaning,
 20 X is a halogen atom, and
 n is an integer from zero to two, inclusive, in a reaction medium comprising, as condensing agent, the compound of Claim 1.

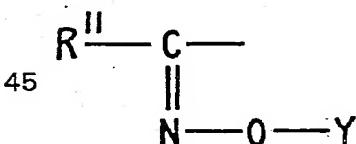
3. An admixture of compounds of the formulae



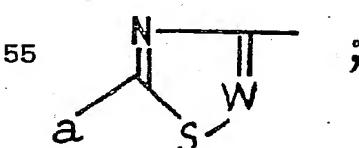
wherein R is α -substituted arylmethyl of the formula



40 or α -oximino of the formula

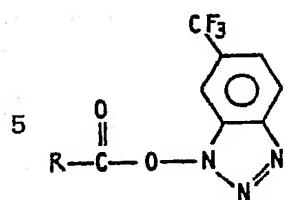


R' is optionally-substituted aryl (homocyclic or heterocyclic);
 50 Q is protected hydroxy, protected amino, protected carboxy or a substitution group in the form of ureido;
 R'' is furyl or



60 a is a hydrogen atom (—H) or —NH₂;
 W is =CH— or =N—; and
 Y is a hydrogen atom (—H), C₁₋₅-alkyl, C₂₋₅-alkenyl, C₂₋₅-alkynyl or cycloalkyl.

4. A compound of the formula

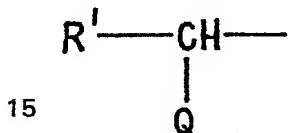


(V)

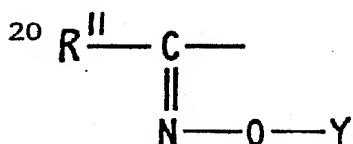
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10 wherein R is α -substituted arylmethyl of the formula

10



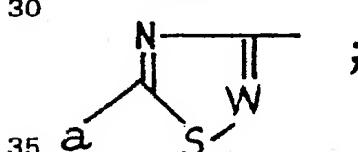
15

or α -oxyimino of the formula

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25 R is optionally-substituted aryl (homocyclic or heterocyclic);
 Q is protected hydroxy, protected amino, protected carboxy or a substitution group in the form
 of ureido;
 R'' is furyl or

25



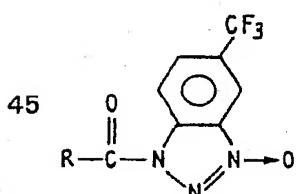
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35 a is a hydrogen atom (-H) or $-\text{NH}_2$;
 W is $=\text{CH}-$ or $=\text{N}-$; and
 Y is a hydrogen atom (-H), C_{1-5} -alkyl, C_{2-5} -alkenyl, C_{2-5} alkynyl or cycloalkyl.

35

40 5. A compound of the formula

40

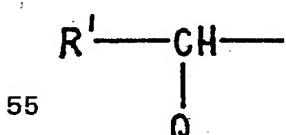


(VI)

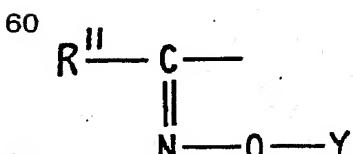
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50 wherein R is α -substituted arylmethyl of the formula

50



55

or α -oxyimino of the formula

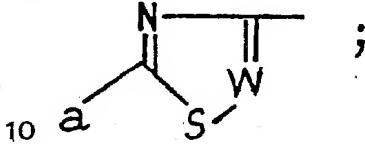
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R' is optionally-substituted aryl (homocyclic or heterocyclic);
Q is protected hydroxy, protected amino, protected carboxy or a substitution group in the form
of ureido;
R'' is furyl or

5



5

a is a hydrogen atom (-H) or -NH₂;
W is =CH- or =N-; and
Y is a hydrogen atom (-H), C₁₋₅-alkyl, C₂₋₅-alkenyl, C₂₋₅-alkynyl or cycloalkyl.

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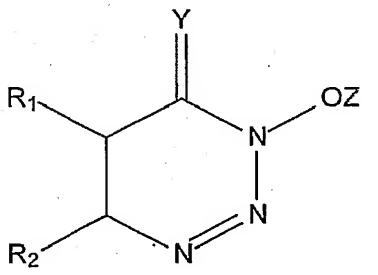
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(54) Title: 3-HYDROXY-4-OXO-1,2,3-TRIAZINES AND DERIVATIVES THEREOF FOR AMIDE AND ESTER BOND FORMATION



(I)

(57) Abstract: The present invention relates to the use of a compound of formula I as a coupling reagent in forming amide or ester bonds from a reaction between a carboxylic acid and an amine or an alcohol, respectively. The compounds of formula I are especially useful as coupling reagents in the preparation of peptide bonds during peptide synthesis. In particular, the compounds of formula I are useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and in suppression of racemization. In addition, the present invention provides novel compounds of Formula I, and salts of N-oxides thereof.

WO 2005/007634 A1

**3-HYDROXY-4-OXO-1,2,3-TRIAZINES AND DERIVATIVES
THEREOF FOR AMIDE AND ESTER BOND FORMATION**

5

FIELD OF THE INVENTION

The present invention relates to a process for forming an amide or ester bond from a reaction between an amine or an alcohol, respectively, and a carboxylic acid or an acylating derivative thereof. More specifically, the invention relates to novel compounds useful as coupling reagents during amide or ester bond formation, for example during peptide synthesis.

BACKGROUND OF THE INVENTION

15

Polypeptides are useful as medicaments. In recent years, peptides have been found to be useful in combating various diseases such as cancer, diabetes, plant toxins and the like. Additionally, peptides have shown specific activity as growth promoters, suppressants, antibiotics, insecticides, contraceptives, anti-hypertensives, sleep-inducers, anti-depressants, 20 analgesics, and other biological functions.

25

Peptides can be synthesized either in solution by classical or various repetitive methods, or on a solid support, so called solid phase peptide synthesis (SPPS) (Merrifield method). These are all popular techniques for synthesizing peptides by coupling two or more amino acids, by coupling amino acids with peptides, or by coupling two or more peptides. Solution methods have the advantage of being easily monitored, allowing purification of intermediates, if necessary, at any stage. A major drawback, however, is the relative slow pace of synthesis, with each step being carried out manually.

30

The major advantage of the Merrifield method is its easy automation so that unattended, computer-controlled machine synthesis is possible. Unfortunately, the method suffers from an inherent deficiency due to the insoluble nature of the support on which the synthesis proceeds. Unless each acylation step occurs with approximately 100% efficiency, mixtures will inevitably be built up on the polymer. The longer the chain, the greater the contamination by undesired side reactions. Side products produced by such reactions remain to contaminate the desired product when it is removed from the polymeric matrix at the end 35 of the cycle.

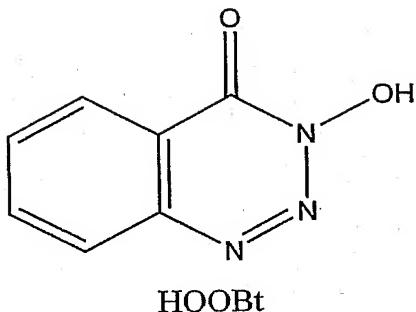
Consequently, these current techniques are not useful in preparing peptides of greater than 40-50 residues, as separation of side products from the desired product becomes increasingly difficult when larger peptides are synthesized.

For very long segments (50 or more amino acids), therefore, current methods (solution phase and SPPS) are less satisfactory. Often, mixtures are obtained of such complexity that it may be difficult or impossible to isolate the desired peptide.

The problems enumerated hereinabove may be eliminated if the proper derivatives of the underlying amino acids and/or the proper conditions for the coupling reaction could be identified. Protecting groups, such as t-butyloxycarbonyl (t-Boc) or N-alpha-(9-fluorenyl methyl)oxycarbonyl (Fmoc), have been used to minimize side reactions. But, additionally, other aspects of the coupling reactions must also be taken into consideration, such as the peptide coupling additive to be used in the coupling reaction.

Additives generally inhibit side reactions and reduce racemization. Currently, the most common peptide coupling additive used during peptide coupling for both solutions and solid phase syntheses is 1-hydroxybenzotriazole (HOEt). This reagent has been used either in combination with a carbodiimide or other coupling agent or built into a stand-alone reagent, such as 1-benzotriazolyoxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), as a phosphonium salt or an analogous uronium salt such as HBTU or TBTU. HOEt is applicable to both stepwise and segment condensations. However, many cases have been encountered in which HOEt is ineffective. In particular, in segment couplings at amino acid units other than glycine or proline the problem of racemization may be severe.

3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HOOEt or HOEt), a related compound, is a well-known reagent for peptide synthesis as an additive for coupling of amino acids in the presence of carbodiimides (Koenig W. et al. DE 1939187 (1971); Koenig W. et al. *Chem. Ber.*, 1970, 103 (7), 2034-40). This compound provides high yield of peptide product and inhibits racemization, especially in difficult coupling and segment condensation (the most racemization sensitive cases in peptide chemistry). In this respect, HOOEt is superior to HOEt and its derivatives, and is comparable in activity to (and in some cases even better than) 1-hydroxy-7-azabenzotriazole (HOAt) (Carpino L.A., El-Faham A., *J. Org. Chem.*, 1995, v.60, p.3561).

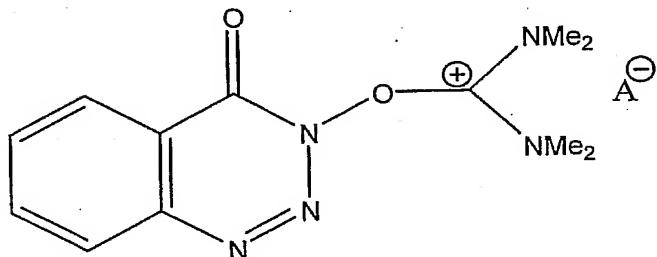


Until recently, the use of HOOBt was much less common than HOAt and HOAt, 5 since the carbodiimide-mediated coupling in the presence of HOOBt is accompanied by formation of a by-product, which itself can react with an N-alpha-amino groups to terminate the chain elongation (Koenig W. et al. *Chem. Ber.*, 1970, 103 (7), 2034-40). It has since then been reported that than the use of particular solvents and conditions can guarantee clean peptide forming reaction in high yield without by-products and racemization (T. Inui, et al. 10 *Letters in peptide Science*, 2002, v.8, p.319; N. Mihala et al. *J. Peptide Science*, 2001, v.7, p. 565; Nozaki S. *J. Pep. Research*, 1999, v.54, p.162).

Several derivatives of HOOBt have been reported as useful additives for amide bond formation. For example, ammonium salts of HOOBt are used as additives for amidation of 15 protected amino acids (Somlai C., et al. *Synthesis*, 1992, p.285); and in peptide synthesis (N. Mihala et al. *J. Peptide Science*, 2001, v.7, p. 565).

Furthermore, the application of HOOBt - active esters of amino acids for peptide synthesis have been reported (Cameron L.R et al. *J. Chem. Soc., Perkin Trans. I.*, 1988, p. 2895; Koenig W. et al. DE 3618218 (1987); Carey R.I. U.S. 5,952,497; Atherton E., et al. *J. Chem. Soc., Perkin Trans. I.*, 1988, p. 2887; Karup G., et al. *Int. J. Peptide Protein Res.*, 20 1988, v.32, p.331).

Carpino et al and Knorr et al disclosed the use of uronium coupling reagents derived from HOOBt, for peptide coupling:

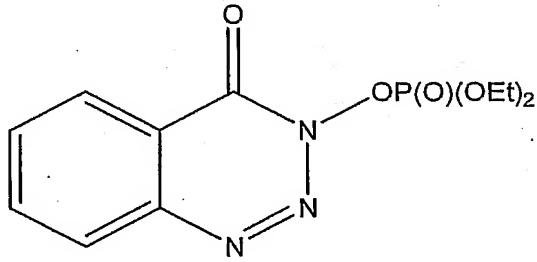


25 A = PF6 (HDTU) (Carpino L.A., El-Faham A., *J. Org. Chem.*, 1995, v.60, p.3561),

BF₄ (TDTU or TDhbTU) (Knorr R., e.a. *Tetrahedron Lett.*, 1989, v.30, p.1927).

However, the use of uronium-salt derivatives as coupling reagents suffers from the drawback of side reactions involving the formation of a guanidine residue at the amino terminus thereby blocking further reactions.

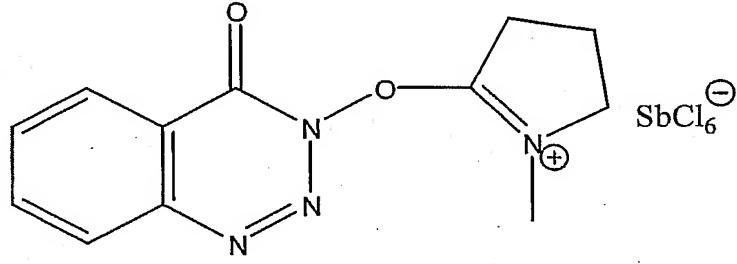
5 A phosphoryloxy derivative of HOOBT, namely 3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT), has been reported. (Li H., et al. *Organic Letters* (1999), 1(1), 91-93; Ye, Yun-Hua et al. *Gaodeng Xuexiao Huaxue Xuebao* (1997), 18(7), 1086-1092; Fan, Chong-Xu et al. *Synthetic Communications* (1996), 26(7), 1455-60).



10

DEPBT

Onium-type peptide coupling agents, such as immonium, pyridinium and thiazolium-coupling reagents, have been reported by Li et al (Li P., Xu J.C. *J. Peptide Res.*, 2001, v.58, p.129). An example is the following HOOBt-derivative immonium-type peptide coupling reagent:



DOMP

20

As polypeptides become of increasing medicinal importance, there is a growing incentive to improve the methods by which they are synthesized. Since HOOBt is a highly efficient coupling reagent, the design and development of novel HOOBt derivatives and analogues, which are effective as peptide coupling additives in both stepwise (batch and

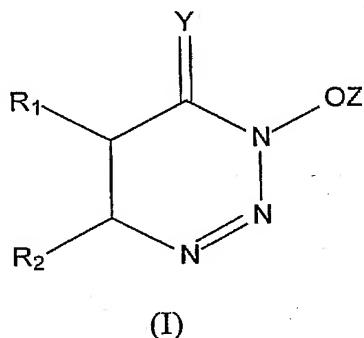
continuous flow) and segment condensations, is highly desirable and urgently needed in the art.

SUMMARY OF THE INVENTION

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The present invention relates to the use of a compound of formula I as a coupling reagent in forming amide or ester bonds from a reaction between a carboxylic acid and an amine or an alcohol, respectively. The compounds of formula I are especially useful as coupling reagents in the preparation of peptide bonds during peptide synthesis. In particular, 10 the compounds of formula I are useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and in suppression of racemization.

In addition, the present invention provides novel compounds of Formula I.



wherein

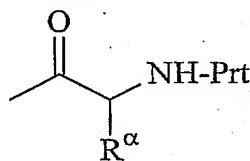
15 Y is O, S or NR₃ wherein R₃ is hydrogen, alkyl or aryl;

R₁ and R₂ are independently of each other selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, halogen, haloalkyl, alkylamino, dialkylamino, arylamino, diarylamino, alkylarylamino, alkoxy, aryloxy, arylalkyloxy, carboxyalkyl, carboxyaryl, carboxyarylalkyl, alkylthio, arylthio, arylalkylthio, cyano, nitro, carbonyl, alkylcarbonyl, arylcarbonyl, arylalkylcarbonyl, alkanoyl, sulfonyl, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, a polymer-based solid support and P(O)(OR₄)₂ wherein R₄ is hydrogen, alkyl or aryl; or R₁ and R₂ together with the carbon atoms to which they are attached form an aryl, heteroaryl, carbocyclic or heterocyclic ring, wherein said aryl, heteroaryl, carbocyclic or heterocyclic ring is optionally substituted with any one or more of the groups R₁ or R₂;

25 Z is selected from

- i) hydrogen;
- ii) P(O)(OR_{4a})₂ wherein R_{4a} is hydrogen, alkyl or aryl;

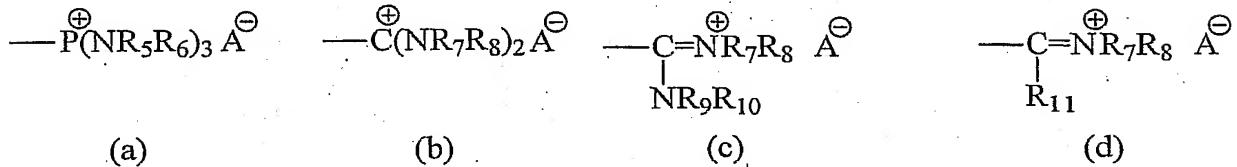
iii) a N-protected amino acid represented by the structure



wherein R^α is an amino acid residue and Prt is a N-protecting group;

iv) $C(O)R_{4b}$ wherein R_{4b} is alkyl or aryl; and

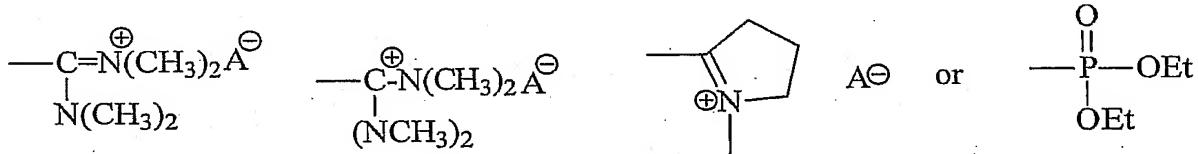
5 v) a group represented by the formula (a), (b), (c), or (d):



10 wherein R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} and R_{11} are independently of each other alkyl, aryl, or cycloalkyl; or one or more of (i) R_5 and R_6 , (ii) R_7 and R_8 , (iii) R_9 and R_{10} , (iv) R_7 and R_{11} , or (v) R_8 and R_{11} , together with the carbon or nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heterocyclic or heteroaryl ring which can optionally contain at least one further nitrogen, oxygen or sulphur in any ring part; and

15 A is PF_6 , BF_4 , Br , Cl , SbF_6 , $SbCl_6$, ClO_4 , $AlCl_4$ or any anion which forms a stable salt, soluble in organic solvents;

with the proviso that when Y is oxygen and Z is hydrogen or one of the groups:



20 R_1 and R_2 together with the carbon atoms to which they are attached are not an unsubstituted phenyl group;

and ammonium salts or N-oxides thereof

In the compounds of formula I, variation of the substituents R_1 , R_2 and Y influences the acidity of the OH group, and thereby influence the effectiveness of the coupling reaction.

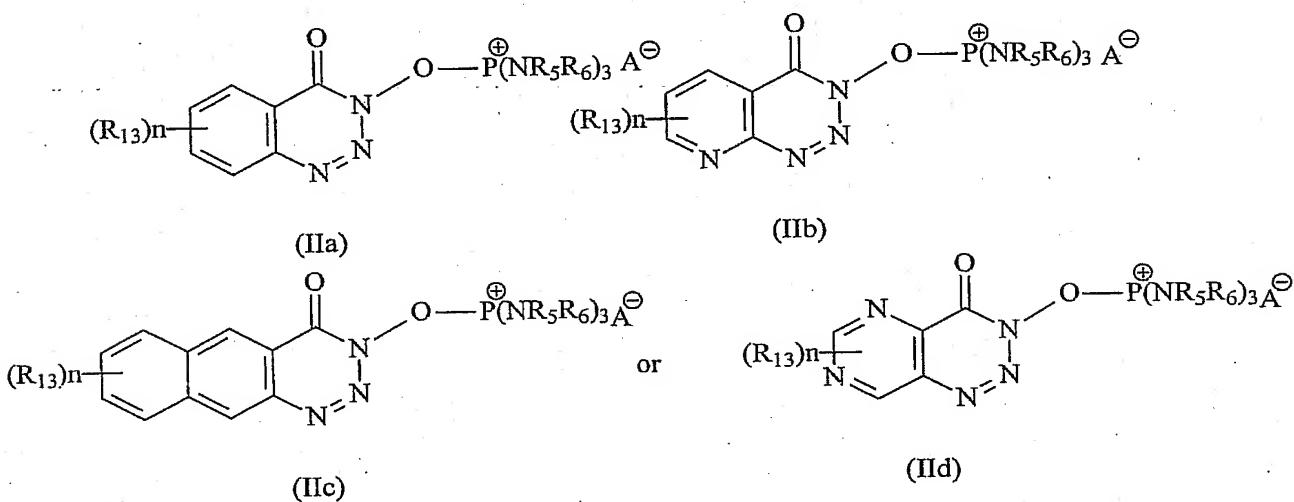
25 In one embodiment of compound I, R_1 and R_2 together with the carbon atoms to which they are attached form a ring selected from the group consisting of phenyl, thiienyl, benzothienyl, 1-naphthothienyl, thianthrenyl, furyl, benzofuryl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, indazolyl,

purinyl, isoquinolyl, quinolyl, naphthyridinyl, quinazolinyl, cinnolinyl, pteridinyl, carbolinyl, isothiazolyl, isoxazolyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclohexadienyl, and cycloheptadienyl.

5 In one embodiment of formula I, Y is O. In another embodiment of compound I, Z is
hydrogen. In another embodiment of formula I, R₁ and R₂ together with the carbon atoms to
which they are attached form an optionally substituted phenyl ring. In another embodiment
of formula I, the compound of formula I is linked to a solid support. The linkage can be for
example through a free hydroxyl or through any other functional group that permits covalent
10 bond formation, such as amino, thiol, carboxyl and the like.

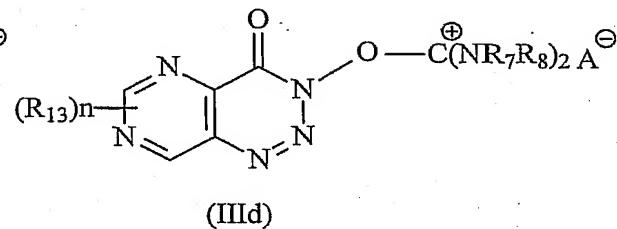
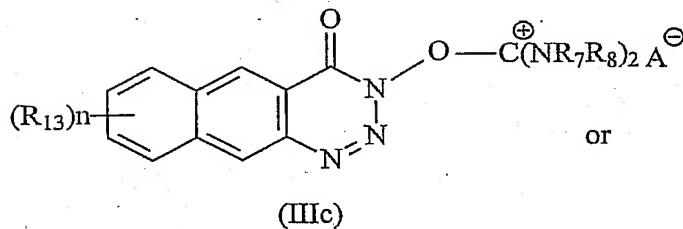
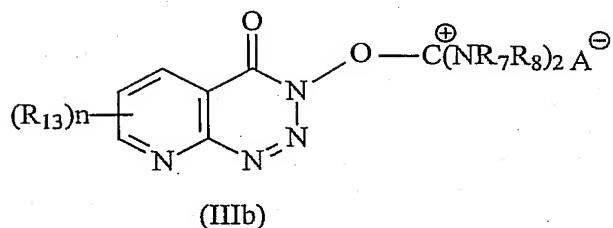
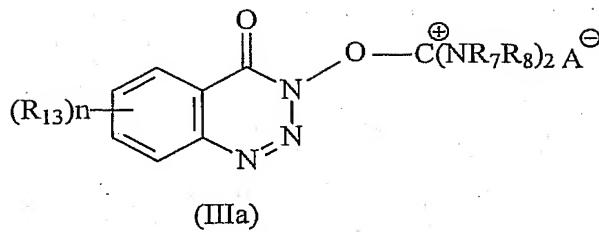
In one embodiment of formula I, A is PF_6^- . In another embodiment, A is BF_4^- .

In another embodiment, the compound of formula I is represented by the structure:



15 wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and
n is 0, 1, 2 or 3.

In another embodiment, the compound of formula I is represented by the structure:

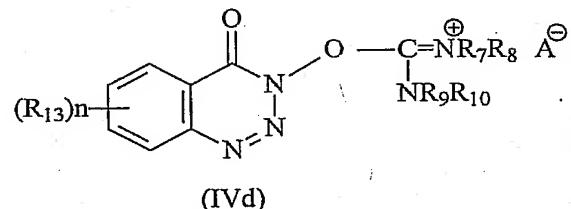
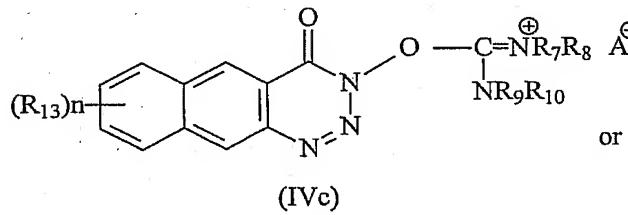
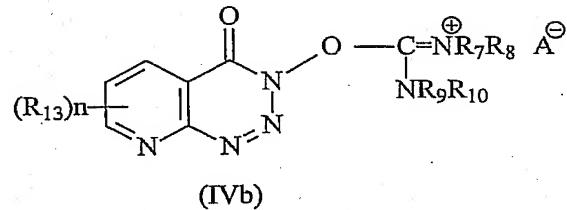
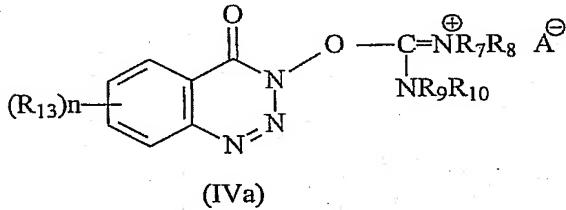


wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

n is 0, 1, 2 or 3;

5 with the proviso that for the compound of formula IIIa, when R_7 and R_8 are both methyl, n is not 0.

In another embodiment, the compound of formula I is represented by the structure:

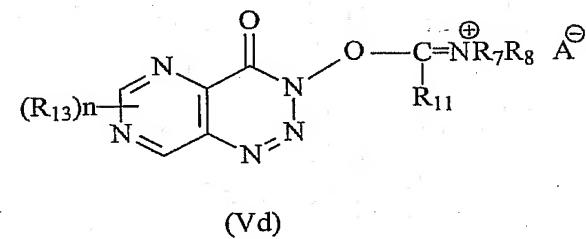
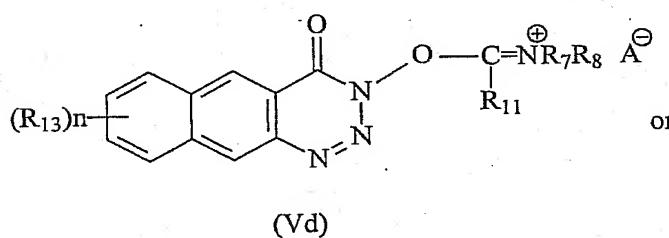
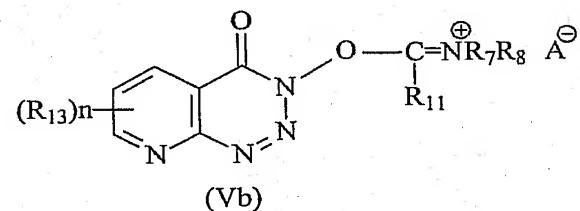
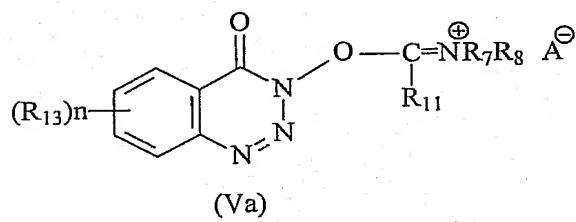


wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

10 n is 0, 1, 2 or 3;

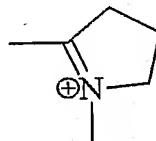
with the proviso that for the compound of formula IVa, when R_7 , R_8 , R_9 and R_{10} are all methyl, n is not 0.

In another embodiment, the compound of formula I is represented by the structure:



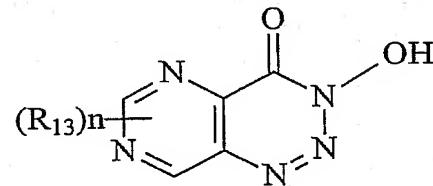
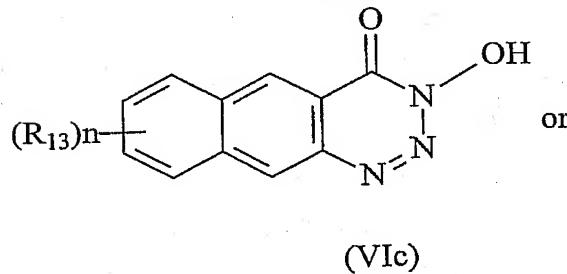
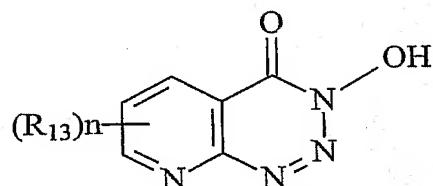
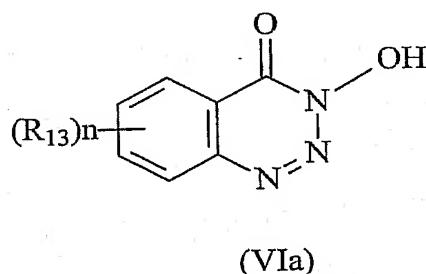
wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

5 n is 0, 1, 2 or 3; with the proviso that when R_7 , R_8 and R_{11} together with the carbon and nitrogen atoms to which they are attached represent a group of the formula:



n is not 0.

10 In another embodiment, the compound of formula I is represented by the structure:

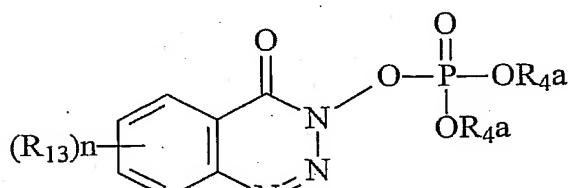


wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

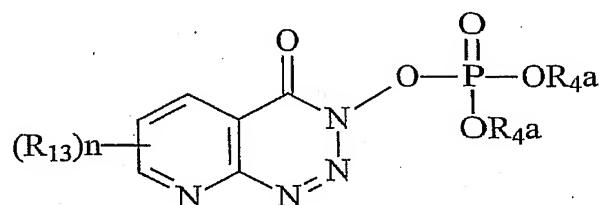
n is 0, 1, 2 or 3.

with the proviso that for the compound of formula VIIa, n is not 0.

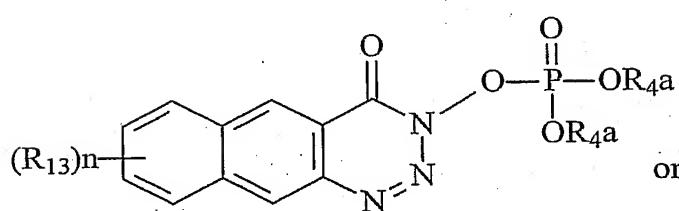
In another embodiment, the compound of formula I is represented by the structure:



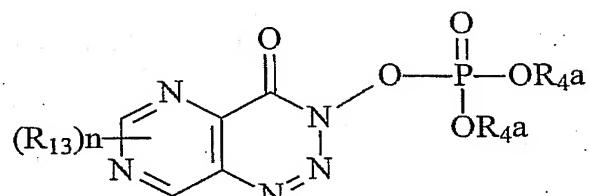
(VIIa)



(VIIb)



(VIIc)



(VIIId)

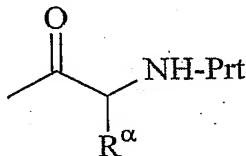
wherein R₁₃ is alkyl, haloalkyl, halogen or NO₂; and

5 n is 0, 1, 2 or 3;

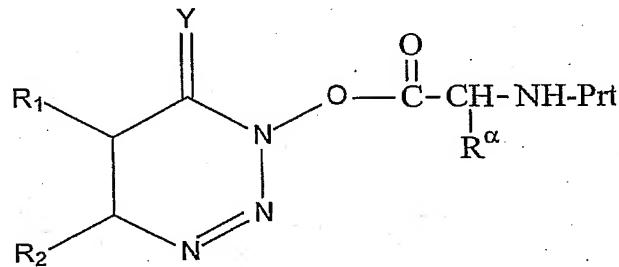
with the proviso that for the compound of formula VIIa, when R_{4a} is ethyl, n is not 0.

In another embodiment, Z is a N-protected amino acid. As used herein, the term "N-protected amino acid" refers to a N- α -amino protected amino acid, or an α -amino acid which bears a protecting group on the amino moiety:

10 In one embodiment, such a N-protected amino acid is represented by the structure:



wherein R ^{α} and Prt are as defined above. In accordance with this embodiment, the compound of formula I is represented by the structure of formula VIII:

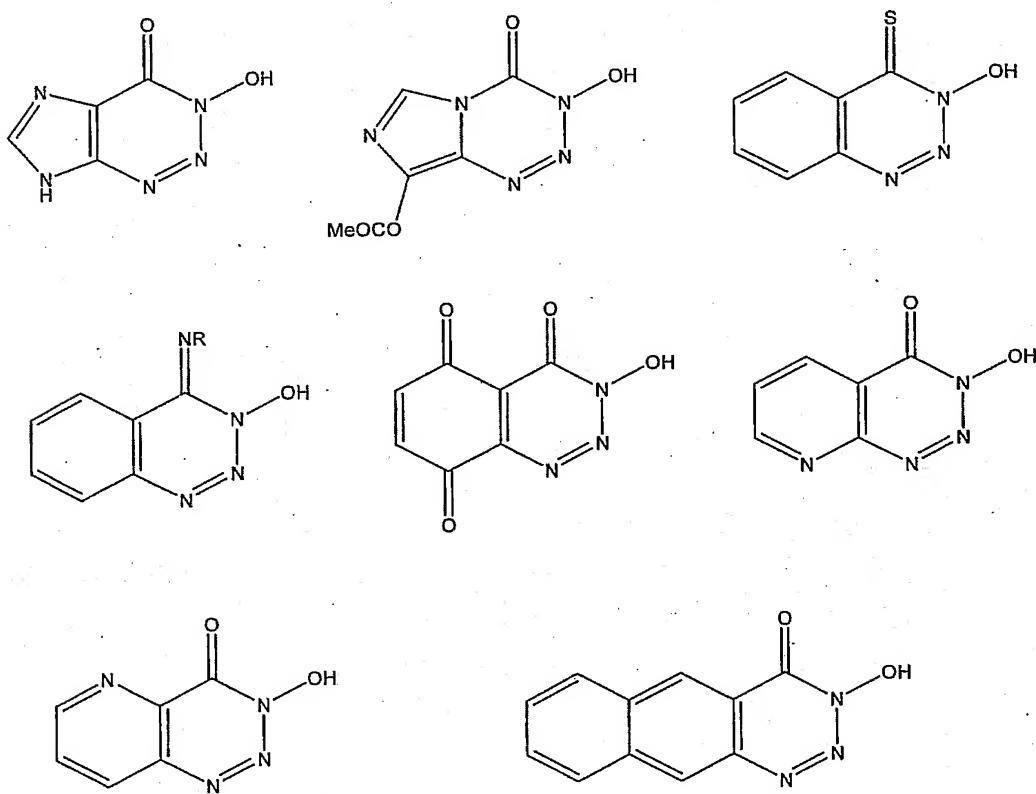


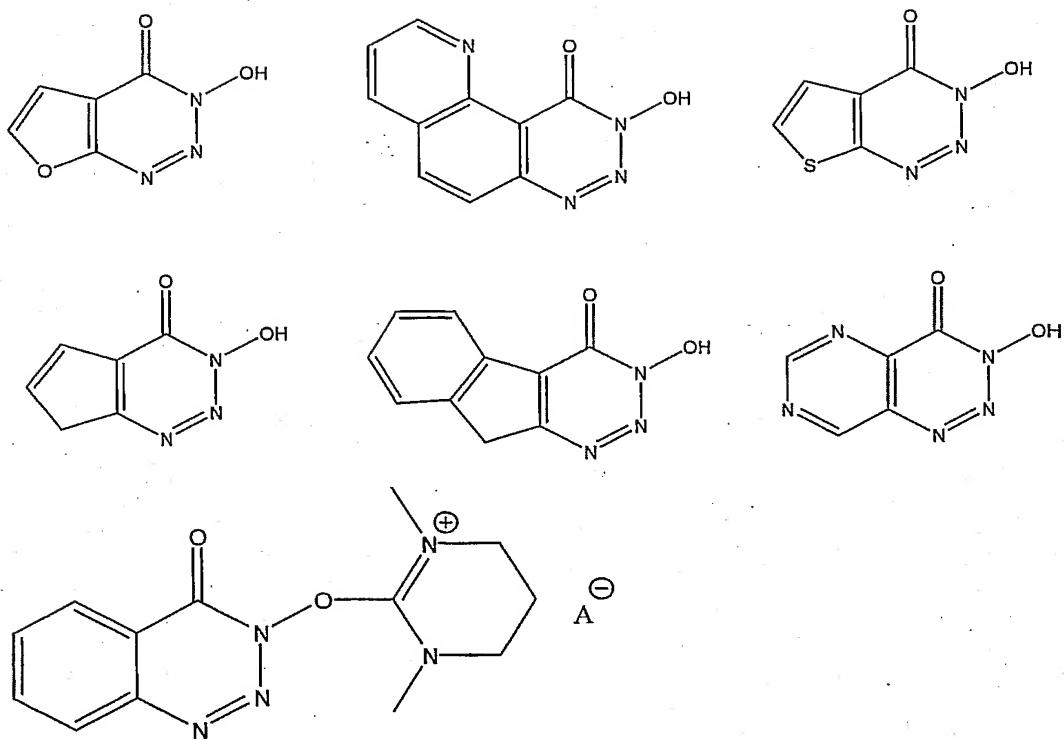
VIII

As contemplated herein, one embodiment of the present invention includes the compound of formula VIII linked to a solid support through either R₁/R₂ or the amino acid residue. Typically, this can be accomplished by coupling to a solid support containing, for example phenyl ester type linkages, thioester linkages or oxime linkages.

5 Specific non-limiting examples of the compound of formula I are provided below. It is apparent to a person skilled in the art that the compounds provided below should not be construed as limiting the broad scope of this invention.

10





5 In another embodiment, the present invention relates to a process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

10 In another embodiment, the present invention relates to a process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of a dehydrating reagent such as EDC or DCC and an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

15 In another embodiment, the present invention relates to a process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of a coupling reagent such as BOP, PyBOP, HBTU or TBTU and an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected. When used as additives, the compounds of formula I

are particularly useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and suppressing racemization.

In another embodiment, the present invention relates to a process for preparing a peptide bond comprising reacting an amino compound with an acylating derivative of a carboxylic acid in the presence of an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected. The reaction can also be conducted in the presence of a dehydrating agent and/or a coupling reagent as defined above.

As defined herein, the term "acylating derivative of a carboxylic acid" refers to a group on the free carboxy end of the amino acid or peptide that facilitates the acylation reaction, i.e., nucleophilic substitution at the acyl carbon. Examples include the free acid, acid halide, anhydride, esters, such as lower alkyl esters, phenoxy esters which are unsubstituted or substituted with 1-5 electron withdrawing groups as defined herein; or an anhydride and the like. The preferred acylating derivative is the acid, acid halide, especially the acid chloride or fluoride, and the phenoxy ester.

In another embodiment, the present invention relates to a process for the synthesis of a peptide, comprising the steps of: a) providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin; b) optionally cleaving the N-protecting group to produce a free amino group; c) coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of an effective amount of a compound of formula I, wherein the carboxyl group is a free carboxyl or an acylating derivative thereof; d) repeating steps (b) and (c) until the desired peptide has been obtained; and e) cleaving the peptide from the resin. The reaction can also be conducted in the presence of a dehydrating agent and/or a coupling reagent as defined above.

In one embodiment, the polypeptide chain is synthesized on an insoluble solid support and the compound of formula I is coupled to the insoluble support. In a particular embodiment, in which the compound of formula I is represented by structural formula III, the compound is linked to the solid support through the amino acid residue. The insoluble support can be for example a phenyl ester-type resin or a resin with oxime or thioester linkages.

The present invention also relates to polymer-supported derivatives the compounds of formula I and their use in peptide synthesis and in organic synthesis.

In another embodiment, the present invention relates to a process for forming an amide bond comprising reacting an organic amine and a carboxylic acid or an acylating derivative thereof in the presence of an effective amount of the compound of formula I. The reaction can also be conducted in the presence of a dehydrating agent and/or a coupling reagent as defined above.

In yet another embodiment, the present invention relates to a process for forming an ester bond comprising reacting an organic alcohol and a carboxylic acid or an acylating derivative thereof in the presence of an effective amount of the compound of formula I. The reaction can also be conducted in the presence of a dehydrating agent and/or a coupling reagent as defined above.

In yet another embodiment, the present invention relates to a process for forming a thioester bond comprising reacting an organic thioalcohol and a carboxylic acid or an acylating derivative thereof in the presence of an effective amount of the compound of formula I. The reaction can also be conducted in the presence of a dehydrating agent and/or a coupling reagent as defined above.

The compounds of Formula I have superior properties relative to the known additives currently in use. Particularly, the compounds of the present invention, as peptide coupling additives, have the ability to accelerate the reactions, provide cleaner processes, higher yields and less racemization, especially in segment or fragment condensations. In addition, the compounds of formula I wherein Z is a phosphonium salt ($P^+(NR_5R_6)3A^-$) as defined above are particularly advantageous, since phosphonium reagents do not take part in guanidine-forming side reactions, as uronium reagents and therefore present an advantage for solid-phase or solution peptide synthesis.

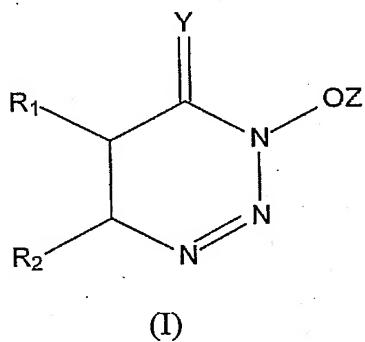
The products formed with the use of compounds of the present invention tend to be purer than those made by methods used until now. In addition, the reaction conditions are very mild, and the reagents used are commercially available and/or easy to prepare.

It is to be understood that any known compounds according to formulae I through VII are excluded explicitly from the scope of the claimed compounds.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention relates to the use of a compound of formula I as a coupling reagent in forming amide or ester bonds from a reaction between a carboxylic acid and an amine or an alcohol, respectively. The compounds of formula I are especially useful as coupling reagents in the preparation of peptide bonds during peptide synthesis. In other words a first amino acid or a first peptide, each having a free amino group is coupled with either a second amino acid or a second peptide having a free carboxyl group or an acylating derivative thereof, in the presence of compounds of Formula I under amide forming conditions to form a peptide bond and thus form a larger peptide. In particular, the compounds of formula I are useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and in suppression of racemization.

In addition, the present invention provides novel compounds of Formula I.



wherein

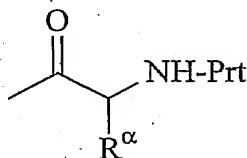
Y is O, S or NR₃ wherein R₃ is hydrogen, alkyl or aryl;

R₁ and R₂ are independently of each other selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, halogen, haloalkyl, alkylamino, dialkylamino, arylamino, diarylamino, alkylarylamino, alkoxy, aryloxy, arylalkyloxy, carboxyalkyl, carboxyaryl, carboxyarylalkyl, alkylthio, arylthio, arylalkylthio, cyano, nitro, carbonyl, alkylcarbonyl, arylcarbonyl, arylalkylcarbonyl, alkanoyl, sulfonyl, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, a polymer-based solid support and P(O)(OR₄)₂ wherein R₄ is hydrogen, alkyl or aryl; or R₁ and R₂ together with the carbon atoms to which they are attached form an aryl, heteroaryl, carbocyclic or heterocyclic ring, wherein said aryl, heteroaryl, carbocyclic or heterocyclic ring is optionally substituted with any one or more of the groups R₁ or R₂;

Z is selected from

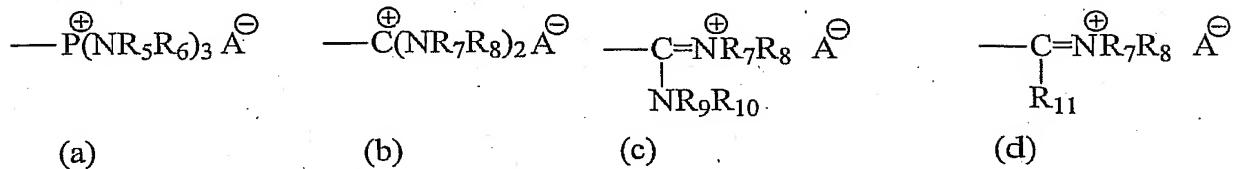
i) hydrogen;

- ii) $P(O)(OR_{4a})_2$ wherein R_{4a} is hydrogen, alkyl or aryl;
- iii) a N-protected amino acid represented by the structure



wherein R^a is an amino acid residue and Prt is a N-protecting group;

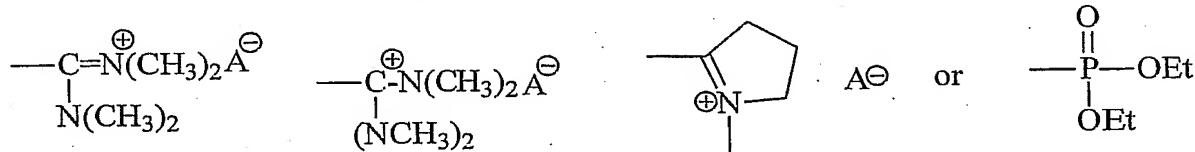
5 iv) $C(O)R_{4b}$ wherein R_{4b} is alkyl or aryl; and
v) a group represented by the formula (a), (b), (c), or (d):



10 wherein R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} and R_{11} are independently of each other alkyl, aryl, or cycloalkyl; or one or more of (i) R_5 and R_6 , (ii) R_7 and R_8 , (iii) R_9 and R_{10} , (iv) R_7 and R_{11} , or (v) R_8 and R_{11} , together with the carbon or nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heterocyclic or heteroaryl ring which can optionally contain at least one further nitrogen, oxygen or sulphur in any ring part;
15 and

A is PF_6^- , BF_4^- , Br^- , Cl^- , SbF_6^- , SbCl_6^- , ClO_4^- , AlCl_4^- or any anion which forms a stable salt, soluble in organic solvents;

with the proviso that when Y is oxygen and Z is hydrogen or one of the groups:



20 R₁ and R₂ together with the carbon atoms to which they are attached are not an unsubstituted phenyl group;

and ammonium salts or N-oxides thereof

In the compounds of formula I, variation of the substituents R_1 , R_2 and Y influences the acidity of the OH group, and thereby influence the effectiveness of the coupling reaction.

25 In one embodiment of compound I, R_1 and R_2 together with the carbon atoms to which they are attached form a ring selected from the group consisting of phenyl, thiienyl, benzothienyl, 1-naphthothienyl, thianthrenyl, furyl, benzofuryl, pyrrolyl, imidazolyl,

pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, indazolyl, purinyl, isoquinolyl, quinolyl, naphthyridinyl, quinazolinyl, cinnolinyl, pteridinyl, carbolinyl, isothiazolyl, isoxazolyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, 5 cyclooctenyl, cyclohexadienyl, and cycloheptadienyl.

In one embodiment of formula I, Y is O. In another embodiment of compound I, Z is hydrogen. In another embodiment of formula I, R₁ and R₂ together with the carbon atoms to which they are attached form an optionally substituted phenyl ring. In another embodiment of formula I, the compound of formula I is linked to a solid support. The linkage can be for example through a free hydroxyl or through any other functional group that permits covalent bond formation, such as amino, thiol, carboxyl and the like.

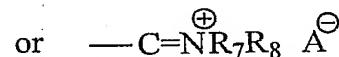
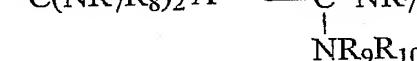
10 In another embodiment, Z is a group represented by the structure (a):



(a)

15 wherein R₅, R₆ and A are as defined above.

In another embodiment, Z is a uronium group represented by the structure (b), (c) or (d):



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(b)

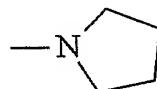
(c)

(d)

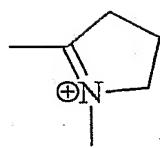
wherein R₇, R₈, R₉, R₁₀, R₁₁, and A are as defined above.

25

In one embodiment, R₇, R₈, R₉, R₁₀ and R₁₁ are independently of each other methyl, ethyl, propyl, butyl, pentyl or phenyl. In another embodiment, R₇ and R₈ are both methyl. In another embodiment, R₉ and R₁₀ are both methyl. In another embodiment, one or more of R₇ and R₈ or R₉ and R₁₀, together with the nitrogen to which they are attached are

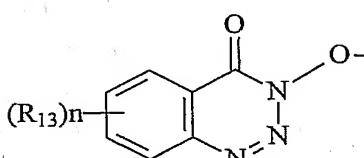


In yet another embodiment, R₇, R₈ and R₁₁ together with the nitrogen and carbon to which they are attached are

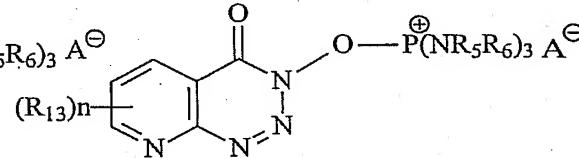


In one embodiment of formula I, A is PF_6^- . In another embodiment, A is BF_4^- .

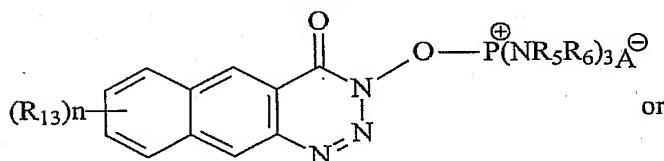
In another embodiment, the compound of formula I is represented by the structure:



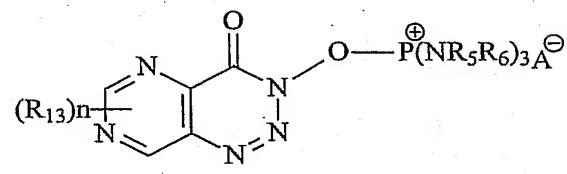
(IIa)



(IIb)



(IIc)

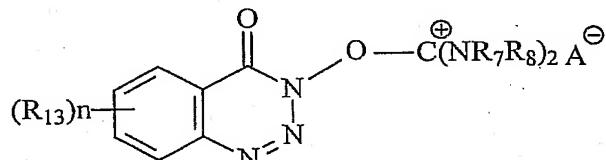


(IId)

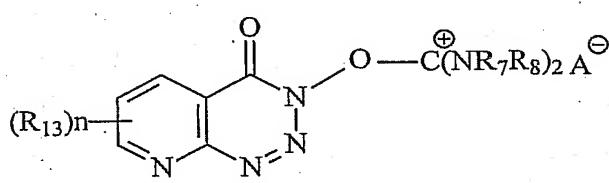
5 wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

n is 0, 1, 2 or 3.

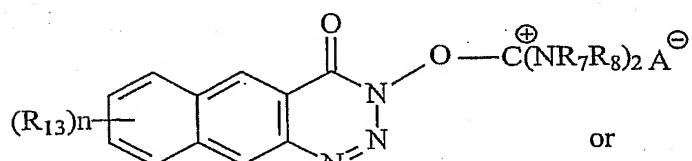
In another embodiment, the compound of formula I is represented by the structure:



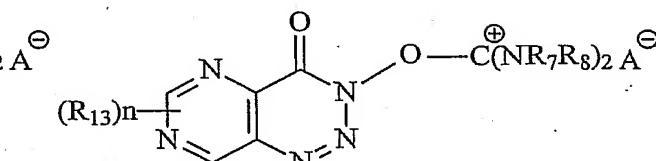
(IIIa)



(IIIb)



(IIIc)



(IIId)

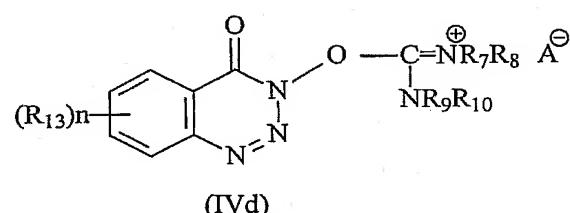
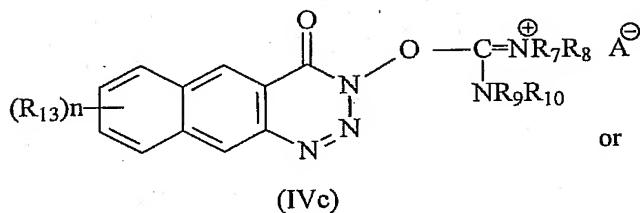
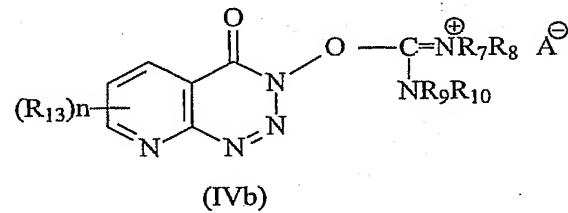
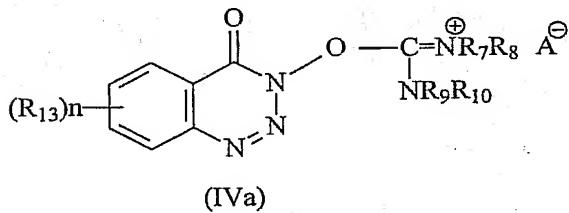
10

wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

n is 0, 1, 2 or 3;

with the proviso that for the compound of formula IIIa, when R₇ and R₈ are both methyl, n is not 0.

In another embodiment, the compound of formula I is represented by the structure:

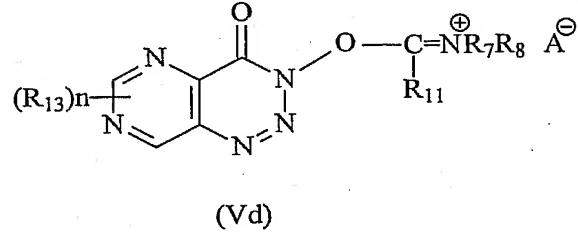
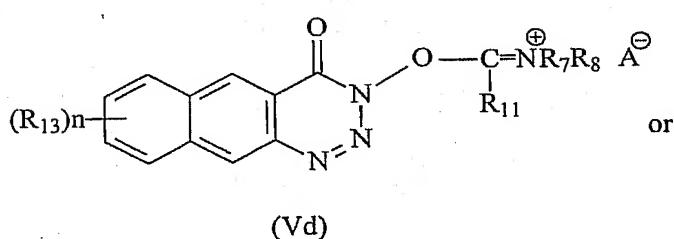
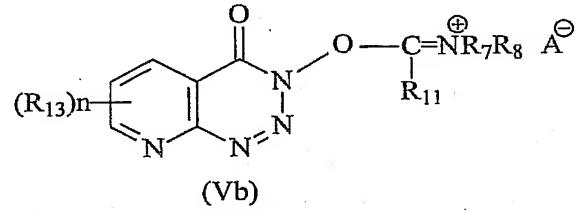
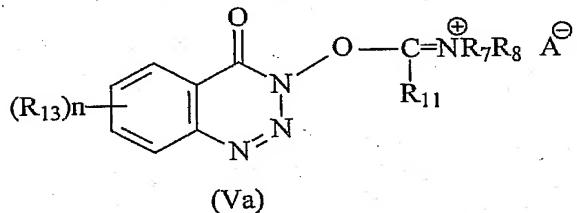


5 wherein R₁₃ is alkyl, haloalkyl, halogen or NO₂; and

n is 0, 1, 2 or 3;

with the proviso that for the compound of formula IVa, when R₇, R₈, R₉ and R₁₀ are all methyl, n is not 0.

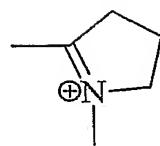
In another embodiment, the compound of formula I is represented by the structure:



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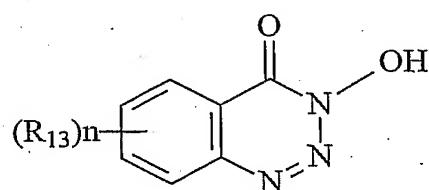
wherein R₁₃ is alkyl, haloalkyl, halogen or NO₂; and

n is 0, 1, 2 or 3; with the proviso that when R₇, R₈ and R₁₁ together with the carbon and nitrogen atoms to which they are attached represent a group of the formula:

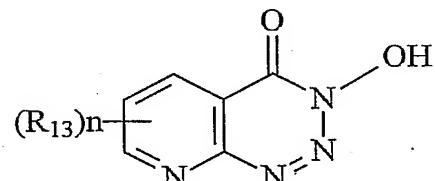


n is not 0.

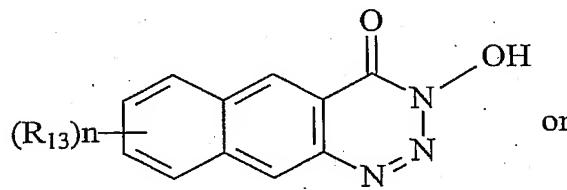
In another embodiment, the compound of formula I is represented by the structure:



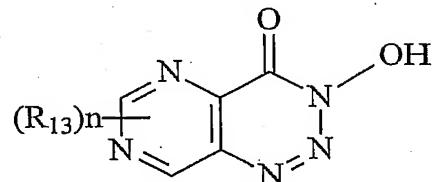
(VIIa)



(VIIb)



(VIIc)



(VID)

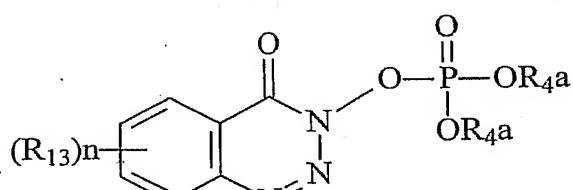
5

wherein R₁₃ is alkyl, haloalkyl, halogen or NO₂; and

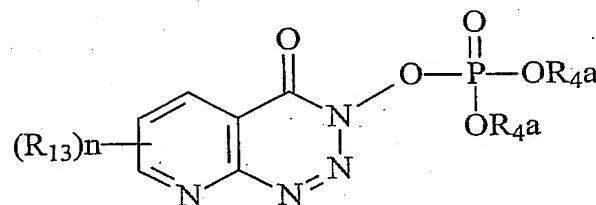
n is 0, 1, 2 or 3.

with the proviso that for the compound of formula VIIa, n is not 0.

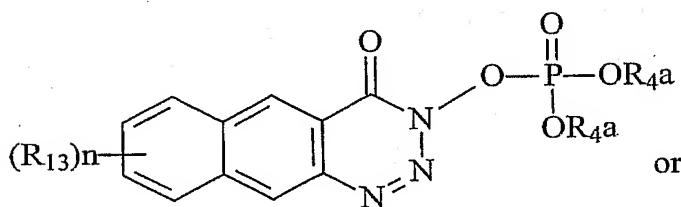
In another embodiment, the compound of formula I is represented by the structure:



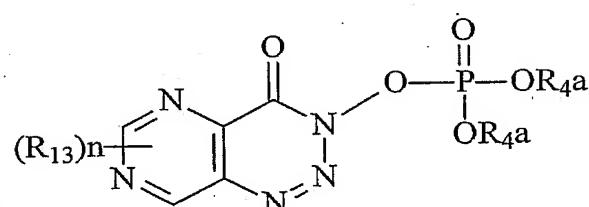
(VIIa)



(VIIb)



(VIIc)



(VIIId)

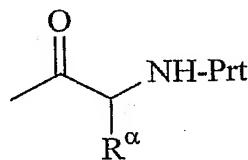
wherein R₁₃ is alkyl, haloalkyl, halogen or NO₂; and

n is 0, 1, 2 or 3;

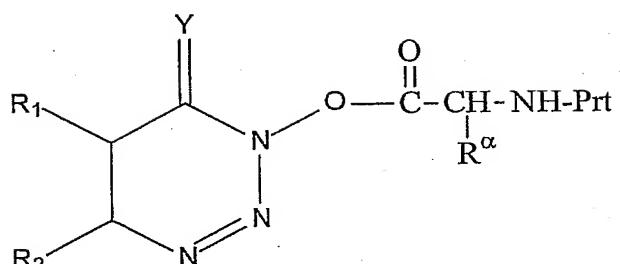
5 with the proviso that for the compound of formula VIIa, when R_{4a} is ethyl, n is not 0.

In another embodiment, Z is a N-protected amino acid. As used herein, the term "N-protected amino acid" refers to a N- α -amino protected amino acid, or an α -amino acid which bears a protecting group on the amino moiety.

10 In one embodiment, such a N-protected amino acid is represented by the structure:



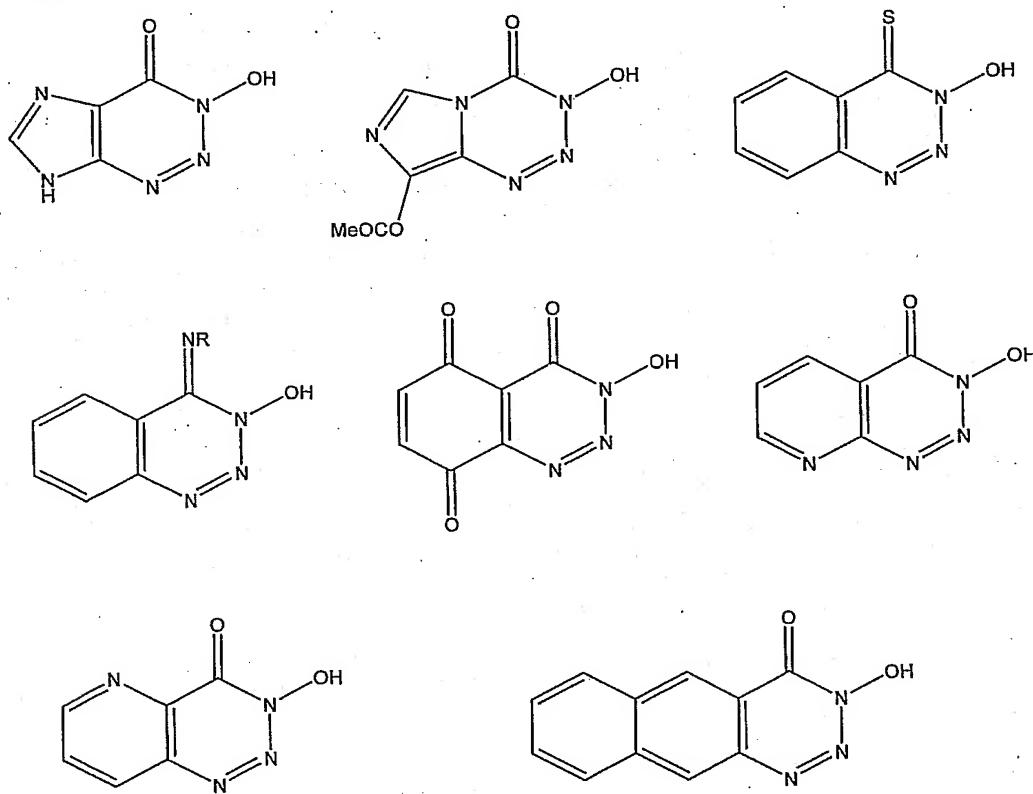
15 wherein R ^{α} and Pr are as defined above. In accordance with this embodiment, the compound of formula I is represented by the structure of formula VIII:

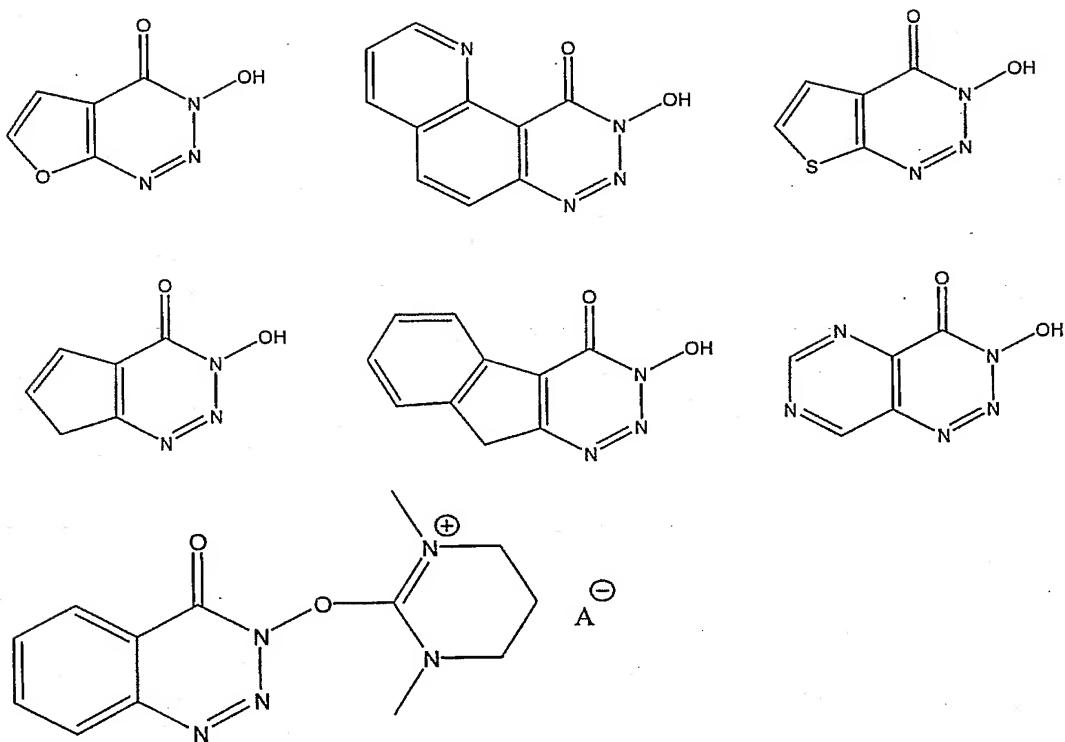


VIII

As contemplated herein, one embodiment of the present invention includes the compound of formula VIII linked to a solid support through either R₁/R₂ or the amino acid residue. Typically, this can be accomplished by coupling to a solid support containing, for example phenyl ester type linkages, thioester linkages or oxime linkages.

Specific non-limiting examples of the compound of formula I are provided below. It is apparent to a person skilled in the art that the compounds provided below should not be construed as limiting the broad scope of this invention.





DEFINITIONS:

5 The term "alkyl" as used herein alone or as part of another group refers to both straight and branched chain hydrocarbons, containing 1 to 20 carbons, preferably 1 to 10 carbons, more preferably 1 to 8 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like and, the various branched chain isomers thereof. Where alkyl groups as defined above have 10 single bonds for attachment to other groups at two different carbon atoms, they are termed "alkylene" groups. The alkyl group can be unsubstituted or substituted through available atoms by one or more of the groups selected from halo for example F, Br, Cl or I, haloalkyl such as CF₃, alkyl, alkoxy, haloalkoxy, trifluoromethoxy, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloheteroalkyl, cycloheteroalkylalkyl, cycloalkenyl, cycloalkenylalkyl, 15 cycloalkynyl, cycloalkynylalkyl, aryl, heteroaryl, arylalkyl, aryloxy, aryloxyalkyl, aryloxyaryl, arylalkyloxy, arylalkenyl, arylalkynyl, arylazo, heteroarylalkyl, heteroarylalkenyl, heteroarylhetereoaryl, heteroaryloxy, hydroxy, hydroxyalkyl, nitro, cyano, amino, alkanoyl, aroyl, alkylamino, dialkylamino, arylamino, diarylamino, thio, alkylthio, arylthio, arylalkylthio, heteroarylthio, alkoxyarylthio, acyl, alkylcarbonyl, arylcarbonyl, 20 alkyl-aminocarbonyl, arylaminocarbonyl, alkoxycarbonyl, aryloxycarbonyl, alkoxycarbonyloxy, aminocarbonyl, alkylaminocarbonyl, arylaminocarbonyl,

alkylcarbonyloxy, arylcarbonyloxy, alkylamido, alkanoylamino, alkylcarbonylamino, arylcarbonylamino, sulfonyl, alkylsulfonyl, arylsulfonyl, aminosulfinyl, sulfonyl, alkylsulfinyl, arylsulfinyl, aminosulfinyl, arylsulfinylalkyl, arylsulfonylamino and aminocarbonyl.

5 The term "alkenyl" as used herein alone or as part of another group refers to straight or branched chain radicals of 2 to 20 carbons, preferably 2 to 12 carbons, and more preferably 1 to 8 carbons in the normal chain, which include one to six double bonds in the normal chain, such as vinyl, 2-propenyl, 3-but enyl, 2-but enyl, 4-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 2-heptenyl, 3-heptenyl, 4-heptenyl, 3-octenyl, 3-nonenyl, 4-decenyl, 3-undecenyl, 10 4-dodecenyl and the like, and which may be optionally substituted with any one or more groups defined hereinabove for alkyl.

15 The term "alkynyl" as used herein alone or as part of another group refers to straight or branched chain radicals of 2 to 20 carbons, preferably 2 to 12 carbons and more preferably 2 to 8 carbons in the normal chain, which include one triple bond in the normal chain, such as 2-propynyl, 3-butyinyl, 2-butynyl, 4-pentynyl, 3-pentynyl, 2-hexynyl, 3-hexynyl, 2-heptynyl, 3-heptynyl, 4-heptynyl, 3-octynyl, 3-nonynyl, 4-dodecynyl and the like, and which may be optionally substituted with any one or more groups defined hereinabove for alkyl.

20 Where alkenyl groups as defined above and alkynyl groups as defined above, respectively, have single bonds for attachment at two different carbon atoms, they are termed "alkenylene groups" and "alkynylene groups", respectively, and may optionally be substituted as defined above for "alkenyl" and "alkynyl".

25 The term "cycloalkyl" as used herein alone or as part of another group refers to a saturated or partially unsaturated (containing 1, 2 or more double bonds), cyclic hydrocarbon ring system containing 1 to 3 rings, including monocyclicalkyl, bicyclicalkyl and tricyclicalkyl and the like, containing a total of 3 to 20 carbons forming the rings, preferably 3 to 10 carbons. Nonlimiting examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, cyclohexenyl, and the like. The cycloalkyl group can optionally be substituted through available carbon atoms with one or more groups defined hereinabove for alkyl.

30 The term "cycloalkenyl" as used herein alone or as part of another group refers to a specific embodiment of "cycloalkyl", and includes partially unsaturated (containing 1, 2 or more double bonds) cyclic hydrocarbons containing 3 to 12 carbons, preferably 5 to 10 carbons and 1, 2, 3 double or more bonds. Nonlimiting examples of cycloalkenyl groups

include cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclohexadienyl, and cycloheptadienyl. The cycloalkyl group can optionally be substituted through available carbon atoms with one or more groups defined hereinabove for alkyl.

5 The term "heterocycloalkyl" (or heterocyclic), as used herein alone or as part of another group refers to a saturated or partially unsaturated ring system containing 1-3 rings, which includes one or more hetero atoms such as nitrogen, oxygen and/or sulfur, such as piperidinyl, piperidinyl, pyrrolidinyl, pyrrolinyl, pyrazolinyl, pyrazolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, pyranyl, thiopyranyl, piperazinyl, indolinyl, dihydrofuranyl, tetrahydrofuranyl, dihydropyranyl, tetrahydropyranyl, and the like. The heterocycloalkyl group can optionally be substituted through available carbon atoms with one or more groups defined hereinabove for alkyl.

10 The term "aryl" as used herein alone or as part of another group refers to an aromatic ring system containing from 6-10 ring carbon atoms and up to a total of 15 carbon atoms. The aryl ring can be a monocyclic, bicyclic, tricyclic and the like. Non-limiting examples of 15 aryl groups are phenyl, naphthyl including 1-naphthyl and 2-naphthyl, and the like. The aryl group can optionally be substituted through available carbon atoms with one or more groups defined hereinabove for alkyl.

15 The term "heteroaryl" as used herein alone or as part of another group refers to a heteroaromatic system containing at least one heteroatom ring atom selected from nitrogen, 20 sulfur and oxygen. The heteroaryl contains 5 or more ring atoms. The heteroaryl group can be monocyclic, bicyclic, tricyclic and the like. Also included in this expression are the benzoheterocyclic Rings. If nitrogen is a ring atom, the present invention also contemplates the N-oxides of the nitrogen containing heteroaryls. Nonlimiting examples of heteroaryls include thienyl, benzothienyl, 1-naphthothienyl, thianthrenyl, furyl, benzofuryl, pyrrolyl, 25 imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, indazolyl, purinyl, isoquinolyl, quinolyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, carbolinyl, isothiazolyl, isoxazolyl and the like. The heteroaryl group can optionally be substituted through available atoms with one or more groups defined hereinabove for alkyl.

30 The term "carbocyclic" as used herein refers to a ring system containing carbon atoms as the only ring atoms. The term refers to monocyclic ring system, as well as bicyclic, tricyclic and the like, and encompasses any of the cycloalkyl or cycloalkenyl substituents defined above.

The term "heterocyclic" as used herein refers to a ring system containing one or more heteroatoms (e.g. N, O, S) as ring agoms. The term refers to monocyclic ring system, as well as bicyclic, tricyclic and the like, and encompasses any of the heterocycloalkyl substituents defined above.

5 The term "halogen" or "halo" as used herein alone or as part of another group refers to chlorine, bromine, fluorine, and iodine. The term "haloalkyl" refers to an alkyl substituent as defined above bearing one or more halogen atoms. An example of a haloalkyl group is a trifluoromethyl group.

10 The term "amino" as used herein alone or as part of another group refers to an NH_2 group. The terms "alkyl amino, dialkylamino, arylamino, diaryl amino" and other substituted amino derivatives as used herein alone or as part of another group refer to amino substituted with one or two substituents, which may be the same or different, such as alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloheteroalkyl, cycloheteroalkylalkyl, cycloalkyl, cycloalkylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, thioalkyl and the like. These 15 substituents can be further substituted with any one or more of the substituents defined above for alkyl. In addition, the amino substituents can be taken together with the nitrogen atom to which they are attached to form a heterocyclic ring such as imidazolyl, pyrrolidinyl, piperidinyl, azepinyl, morpholinyl, thiomorpholinyl, piperazinyl, and the like.

20 The term "hydroxy" refers to an OH group. The terms "alkoxy", "aryloxy" or "arylalkyloxy" as used herein alone or as part of another group includes any of the above alkyl, arylalkyl or aryl groups linked to an oxygen atom.

25 The term "carboxy" as used herein alone or as part of another group refers to a COO group, and further encompasses carboxylate salts thereof of the formula COOM wherein M is a metal ion. The term "metal ion" refers to alkali metal ions such as sodium, potassium or lithium and alkaline earth metal ions such as magnesium and calcium, as well as zinc and aluminum. The term "carboxyalkyl", "carboxyaryl" or "carboxyarylalkyl" as used herein alone or as part of another group includes any of the above alkyl, arylalkyl or aryl groups linked to a carboxy group.

30 The term "thio" as used herein alone or as part of another group refers to an SH group. The terms "alkylthio", "arylthio" or "arylalkylthio" as used herein alone or as part of another group refer to any of the above alkyl, arylalkyl or aryl groups linked to a sulfur atom.

The term "cyano" as used herein alone or as part of another group refers to a CN group. The term "nitro" as used herein alone or as part of another group refers to an NO₂ group.

5 The term "carbonyl" as used herein alone or as part of another group refers to a C=O group. The terms "alkylcarbonyl", "arylcarbonyl" or "arylalkylcarbonyl" as used herein alone or as part of another group refer to any of the above alkyl, arylalkyl or aryl groups linked to a carbonyl group.

The term "alkanoyl" as used herein alone or as part of another group refers to an alkyl linked to a carbonyl group.

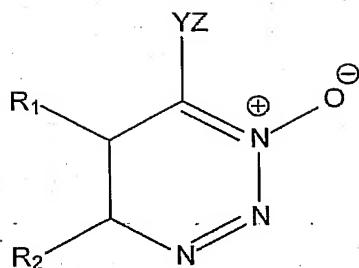
10 The term "sulfonyl" as used herein alone or as part of another group refers to an SO₂ group. The terms "alkylsulfonyl", "arylsulfonyl" or "arylalkylsulfonyl" as used herein alone or as part of another group refer to any of the above alkyl, arylalkyl or aryl groups linked to a sulfonyl group.

15 The term "acyl" as used herein alone or part of another group, refers to an organic radical linked to a carbonyl. Nonlimiting examples of acyl groups are alkanoyl, alkenoyl, aroyl, aralkanoyl, heteroaroyl, cycloalkanoyl, cycloheteroalkanoyl and the like.

20 Where the compounds of structure I are in acid form it may form a pharmaceutically acceptable salt such as alkali metal salts such as lithium, sodium or potassium, alkaline earth metal salts such as calcium or magnesium as well as zinc or aluminum and other cations such as ammonium, choline, diethanolamine, ethylenediamine, t-butylamine, t-octylamine, dehydroabietylamine and the like.

25 All stereoisomers of the compounds of the instant invention are contemplated, either in admixture or in pure or substantially pure form. The compounds of the present invention can have asymmetric centers at any of the carbon atoms including any one or the R substituents. Consequently, compounds of formula I can exist in enantiomeric or diastereomeric forms or in mixtures thereof. The compounds can be racemates, enantiomers or diastereomers as starting materials. When diastereomeric or enantiomeric products are prepared, they can be separated by conventional methods for example, chromatographic or fractional crystallization.

30 In addition, the compounds of formula I can exist in tautomeric forms or in mixtures thereof, which have the same reactivity as compound (I) and are also useful use as coupling reagents in forming amide or ester bonds. An example of such a compound is compound of formula:



5 In addition, when the compounds of formula I contain double bonds, all geometrical isomers such as cis, trans, E and Z are contemplated.

When R₁ and R₂ taken together with the carbons to which they are attached form a tricyclic group, then the compounds of Formula I is tetracyclic. Similarly, if a bicyclic group is formed from R₁ and R₂ taken together with the carbons to which they are attached, then the compounds of Formula I are tricyclic. Finally, if R₁ and R₂ taken together form a 10 monocyclic group, then the compounds of Formula I are bicyclic.

Depending on the definition of R₁ and R₂ (electron donating or electron withdrawing), the acidity of the OH or phosphonium group can be influenced and thereby the effectiveness of coupling.

As used herein, an "electron donating group" refers to a group that will release or 15 donate electrons more than hydrogen would if it occupied the same position in the molecule. See J. March, Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons p. 238 (1985). These types of groups are well known in the art. Examples include alkylamino, amino, halo, aryl, alkoxy, aralkoxy, aryloxy, mercapto, alkylthio, and the like.

The term "electron withdrawing groups" as defined herein refers to a group that will 20 draw electrons to itself more than a hydrogen atom would if it occupied the same position in the molecule. See, J. March, Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons P. 17 (1985). They include such groups as nitro, monohaloalkyl, dihaloalkyl, trihaloalkyl (e.g., CF₃), halo, formyl, lower alkanoyl, lower alkylsulfonyl, lower alkylsulfinyl, and the like.

25 Of course, various combinations and permutations of the formulae described herein are also contemplated by the present invention. In addition, Markush groupings containing less than all of the elements described hereinabove as well as the various permutations thereof are also contemplated by the present invention.

As described herein, the compounds described hereinabove are useful in promoting peptide coupling, i.e., the reaction between a free amino group of a first amino acid or first peptide with a free carboxy group or acylating derivative thereof of a second amino acid or peptide. The process of the present invention is general; it can be used in effecting the 5 coupling of a dipeptide and an amino acid, a tripeptide and an amino acid, a tetrapeptide and an amino acid, dipeptides, pentapeptides, higher peptides, polypeptides etc.

Accordingly, the present invention provides, in one embodiment, a process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is 10 optionally N-protected or a peptide which is optionally N-terminal amino protected.

In one embodiment, the coupling reactions described hereinabove can take place in the additional presence of a dehydrating reagent such as DCC (dicyclohexylcarbodiimide) or EDC, (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride) and the like. In 15 accordance with this embodiment, the present invention provides a process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of a dehydrating agent and an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

In another embodiment, the coupling reactions described hereinabove can take place in the additional presence of a coupling reagent such as BOP, PyBOP, HBTU or TBTU. When used as additives, the compounds of formula I are particularly useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and suppressing racemization. In accordance with this embodiment, the present invention provides a process 20 for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of a coupling agent and an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

In yet another embodiment, the process of the present invention comprises using an acylating derivative of a carboxylic acid for the coupling reaction. In accordance with this 30 embodiment, the present invention provides a process for preparing a peptide bond comprising reacting an amino compound with an acylating derivative of a carboxylic acid in

the presence of an effective amount of a compound of formula I; wherein the amino compound is an amino acid or peptide and the carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected. This coupling reaction can be conducted in the presence of the compound of formula I alone, or 5 with the further addition of a dehydrating agent and/or a coupling reagent as described above.

As defined herein, the term "acylating derivative of a carboxylic acid" refers to a group on the free carboxy end of the amino acid or peptide that facilitates the acylation reaction, i.e., nucleophilic substitution at the acyl carbon. Examples include the free acid, 10 acid halide, anhydride, esters, such as lower alkyl esters, phenoxy esters which are unsubstituted or substituted with 1-5 electron withdrawing groups as defined herein; or an anhydride and the like. The preferred acylating derivative is the acid, acid halide, especially the acid chloride or fluoride, and the phenoxy ester.

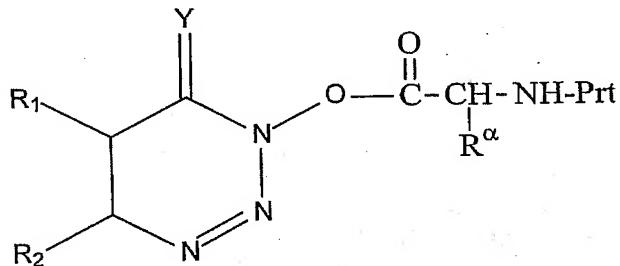
A preferred acylating group of an amino acid is the amino acid chloride or fluoride. 15 The preparation and use of amino acid chlorides as an acylating derivative is discussed in an article by Carpino, et al. in *J. Org. Chem.*, 1986, 51, 3734-3736, the contents of which are incorporated herein by reference. Briefly, amino acid chlorides can be prepared by reacting the amino acid with thionyl chloride and recrystallizing the product from a recrystallization reagent, such as CH_2Cl_2 -hexane.

20 Amino acid fluorides can be prepared by reacting an N-protected amino acid with the reagent cyanuric fluoride. This reaction can be run at temperatures as low as 0°C . and up to the refluxing temperature of the solvent, but it is preferred that the reaction is run at room temperature. It can also be run in an inert solvent, such as pyridine, CH_2Cl_2 and the like. The cyanuric fluoride can be prepared from the corresponding chloride in the presence of 25 potassium fluoride at elevated temperatures. Other fluorinating agents well known in the art, such as thionyl fluoride, 2,4,6-trinitrofluorobenzene, N-methyl-2-fluoropyridinium salts, and the like may be used in place of KF to effect the formation of cyanuric fluoride.

Another embodiment, the present invention relates to a process for the synthesis of a peptide, comprising the steps of: a) providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin; b) optionally cleaving the N-protecting group to produce a free amino group; c) coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of an effective amount of a compound 30

of formula I, wherein the carboxyl group is a free carboxyl or an acylating derivative thereof; d) repeating steps (b) and (c) until the desired peptide has been obtained; and e) cleaving the peptide from the resin. This coupling reaction can be conducted in the presence of the compound of formula I alone, or with the further addition of a dehydrating agent and/or a coupling reagent as described above. In addition, a carboxylic acid having a free carboxyl group can be used, or an acylating derivative of the carboxylic acid, as defined hereinabove.

In one embodiment, the polypeptide chain is synthesized on an insoluble solid support and the compound of formula I is coupled to the insoluble support. In a particular embodiment, in which the compound of formula I is represented by structural formula VIII, the compound is linked to the solid support through the N-protected amino acid residue or through one of the residues R₁ and/or R₂. The insoluble support can be for example a phenyl ester-type resin or a resin with oxime or thioester linkages



In another embodiment, the present invention provides a process for forming an amide bond comprising reacting an organic amine with a carboxylic acid in the presence of an effective amount of the compound of formula I. This reaction can be conducted in the presence of the compound of formula I alone, or with the further addition of a dehydrating agent and/or a coupling reagent as described above. In addition, a carboxylic acid having a free carboxyl group can be used, or an acylating derivative of the carboxylic acid, as defined hereinabove.

In yet another embodiment, the present invention relates to a process for forming an ester bond comprising reacting an organic alcohol and a carboxylic acid in the presence of an effective amount of the compound of formula I. This reaction can be conducted in the presence of the compound of formula I alone, or with the further addition of a dehydrating agent and/or a coupling reagent as described above. In addition, a carboxylic acid having a free carboxyl group can be used, or an acylating derivative of the carboxylic acid, as defined hereinabove.

The coupling reactions usually take place in an inert organic solvent such as dimethylformamide (DMF), N-methylpyrrolidone (NMP), acetonitrile, methylene chloride or ethers, such as THF or dioxane or mixtures of solvents. In fact, DMF is the preferred solvent in the solid phase synthesis because of its favorable solvation properties. The reactions take 5 place under mild conditions usually ranging from about 0°C to about 30°C.

The term "amino acid" as used herein refers to an organic acid containing both a basic amino group (NH₂) and an acidic carboxyl group. (COOH). Therefore, said molecule is amphoteric and exists in aqueous solution as dipole ions. (See "The Condensed Chemical Dictionary", 10th Ed., edited by Gessner G. Hawley, Van Nostrand Reinhold Company, 10 London, England p. 48 (1981)). The preferred amino acids are the α -amino acids. They include but are not limited to the 25 amino acids that have been established as protein constituents. They must contain at least one carboxyl group and one primary or secondary amino group in the amino acid molecule. The term includes natural as well as unnatural amino acids, such as such alanine, valine, leucine, isoleucine, norleucine, proline, 15 hydroxyproline, phenylalanine, tyrosine, tryptophan, methionine, glycine, serine, homoserine, threonine, cysteine, cystine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, hydroxylysine, ornithine, arginine, homoarginine, histidine, penicillamine, naphthylamine, alpha-phenylglycine, isoglutamine, pyroglutamic acid, gamma-aminobutyric acid, citrulline, sarcosine, statine and the like.

20 As used herein, the term "peptide" refers to the class of compounds composed of amino acid units chemically bound together with amide linkages. A peptide may contain as little as two amino acid residues or may contain a polymer of amino acid residues (polypeptide).

As used herein, the terms "amino acid" and "peptide" also include amino acids and peptides, respectively containing blocking (protecting) groups. These protecting "groups" 25 (abbreviated "Prt" in the compounds of formula I) block the amino group or the carboxyl group of the amino acid or peptide not involved in or taking part in the coupling in order to prevent unwanted side reactions. These protecting groups also protect reactive groups on the side chain. After the peptide is formed, the blocking groups are removed by techniques known to one skilled in the art.

30 A number of blocking reagents for amino groups are known in the art and have been utilized in the syntheses of peptides. These blocking groups are discussed in U.S. Pat. Nos. 3,835,175, 4,508,657, 3,839,396, 4,581,167, 4,394,519, 4,460,501 and 4,108,846, the contents of all of which are incorporated by reference as if fully set forth herein. Other amino

protecting groups are described in an article entitled "Solid Phase Peptide Synthesis", by G. Barany and R. B. Merrifield in THE PEPTIDES, Vol. 2, edited by E. Gross and J. Meienhoffer, Academic Press, N.Y., N.Y. 100-118 (1980), and in the book entitled "PROTECTIVE GROUPS IN ORGANIC SYNTHESIS" by T. W. Green, John Wiley & Sons, New York, the contents of all of which are being incorporated by reference.

5 Amino protecting groups ("N-protecting group", "Prt", or "N- α -amino protecting group", used herein interchangeably) as used herein, refers to blocking groups which are known in the art and which have been utilized to block the amino (NH_2) group of the amino acid. Blocking groups such as 9-lower alkyl-9-fluorenyloxycarbonyl-2-chloro-1-indanylmethoxy-carbonyl (CLIMOC) and benz [f] indene-3methyloxycarbonyl (BIMOC) and dbd-TMOC are discussed in U.S. Pat. Nos. 3,835,175, 4,508,657, 3,839,396, 4,581,167, 10 4,394,519, 4,460,501 and 4,108,846 referred to hereinabove. Other N-amino protecting groups include such groups as the t-butyloxycarbonyl (BOC), t-amyoxy carbonyl (Aoc), β -trimethylsilyl-ethyloxycarbonyl (TEOC), adamantyl-oxycarbonyl (Adoc), 15 1-methylcyclobutyloxycarbonyl (Mcb), 2-(p-biphenylyl)propyl-2-oxycarbonyl (Bpoc), 2-(p-phenylazophenyl)propyl-2-oxycarbonyl (Azoc), 2,2-dimethyl-3,5-dimethyloxymethyl oxycarbonyl (Ddz), 2-phenylpropyl-2oxycarbonyl (Poc), benzyloxycarbonyl (Cbz), p-toluenesulfonyl aminocarbonyl (Tac), o-nitrophenylsulfenyl (Nps), dithiasuccinoyl (Dts), Phthaloyl, piperidineoxycarbonyl, formyl, trifluoroacetyl and the like.

20 These protecting groups can be placed into five categories:

- 1) a base labile N- α -amino acid protecting group such as FMOC, and the like;
- 2) protecting groups removed by acid, such as Boc, TEOC, Aoc, Adoc, Mcb, Bpoc, Azoc, Ddz, Poc, Cbz, 2-furanmethyloxycarbonyl (Foc), p-methoxybenzyloxycarbonyl (Moz), Nps, and the like;
- 25 3) protecting groups removed by hydrogenation such as Dts, Cbz;
- 4) protecting groups removed by nucleophiles, such as Bspoc, Bsmoc and Nps and the like; and
- 5) protecting groups derived from carboxylic acids, such as formyl, acetyl, trifluoroacetyl and the like, which are removed by acid, base or nucleophiles.

30 A variety of carboxy protecting groups known in the art may be employed. Examples of many of these possible groups may be found in "Protective Groups in Organic Synthesis", by T. W. Green, John Wiley & Sons, 1981, the contents of which is incorporated by

reference. These examples include such groups as methyl ester, t-butyl ester, beta-trimethylsilylethyl ester, benzyl ester and the like.

In addition, during the course of protein synthesis, it may be necessary to protect certain side chains of the amino acids to prevent unwanted side reactions. For example, the following amino acids contain functional groups that can be protected: arginine, lysine, aspartic acid asparagine, glutamic acid, glutamine, histidine, cysteine, ornithine, serine, threonine, homoarginine, citrulline and tyrosine. The protecting groups can be any of the nitrogen or carboxyl protecting groups described hereinabove, and are also set forth in "Solid Phase Peptide Synthesis", by G. Barany and R. B. Merrifield in THE PEPTIDES, Vol. 2, edited by E. Gross and J. Meienhoffer, Academic Press, N.Y., N.Y. 100-118 (1980), and in the book entitled "PROTECTIVE GROUPS IN ORGANIC SYNTHESIS" by T. W. Green, John Wiley & Sons, New York, the contents of all of which are being incorporated by reference.

A typical preparation of the peptide in accordance with the present invention involves the following steps

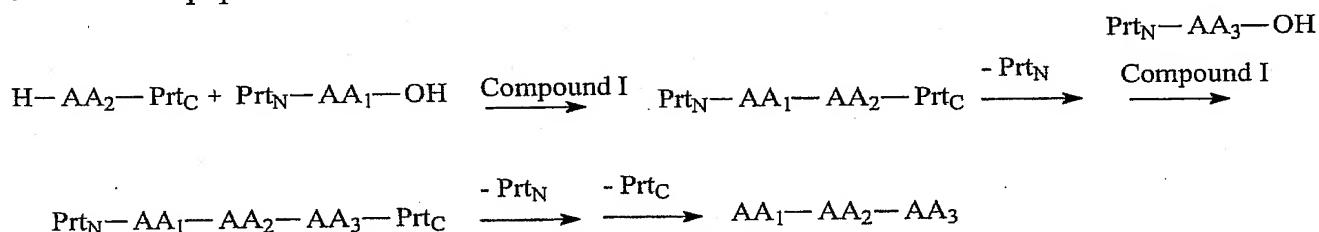
- 1) Protection of the free carboxyl group in a first amino acid or a first peptide, or anchoring the amino acid or peptide to a solid support;
- 2) Protection of the free amino group of a second amino acid or peptide;
- 3) Protection of the side chains, if necessary;
- 20 4) Coupling the first amino acid or peptide with the second amino acid or peptide in the presence of compounds of Formula I;
- 5) Deprotecting the amino protecting group to produce a free amino group;
- 6) Repeating steps 2-5 until the desired peptide has been obtained;
- 7) Cleaving the peptide from the resin, and
- 25 8) Removal of the protecting groups.

The procedure of steps 1-3 can be performed in any order. In addition, the procedure of steps 1-3 can be performed in any order.

In the coupling step, the compounds of Formula I should be present in effective amounts. Usually, the first amino acid or peptide is present in approximately equimolar amounts with the second amino acid or peptide, although the reaction can take place if the molar ratio of the former to the latter ranges from 1:3 to 3:1. Furthermore, the amount of the compound having Formula I used depends upon the amount of peptide or amino acid which is present in the least amount (i.e. the limiting reagent); thus the molar ratio of the compound

of Formula I to the amino acid or peptide ranges from 1:3 to 3:1 relative to the amino acid or peptide present in the least molar amount, although it is preferred that approximately equimolar amounts of the compound of Formula I, the first amino acid or peptide and the second amino acid or peptide be used. In some cases, the molar ratio of compound I and the carboxylic acid in the range of 0.02 to 0.10 is also effective.

The following sequence (Scheme 1) is illustrative of the coupling reaction; in the examples below, amino acids (AA) are used, although the procedure is general for amino acids and/or peptides:



10

Scheme 1

In the above scheme, "Prt_N" is an amino acid blocking group, "Prt_C" is a carboxylic acid blocking group, and AA₁, AA₂ and AA₃ are first, second and third amino acid, respectively.

15 As shown by the above scheme, the N- α -amino protected amino acid is reacted with a second amino acid in which the carboxy group is protected.

A peptide is formed between the first amino acid and the second amino acid in the presence of a compound of formula I. The peptide chain can be increased by removing the amino protecting group by techniques known to one skilled in the art and then reacting the corresponding dipeptide with another N- α -amino protected amino acid in the presence of a compound of Formula I to form the corresponding tri-peptide. The N- α amino protecting group of the tri-peptide is removed and the above-cycle is repeated until the desired peptide has been obtained.

25 The present invention can readily be utilized in solid phase peptide synthesis. Solid phase peptide synthesis is based on the stepwise assembly of a peptide chain while it is attached at one end to a solid support or solid phase peptide resin. Two methods are generally well known in the art.

One, the Merrifield method, employs a solid support for attachment of the amino acid or peptide residues. This method employs N-protected amino acids as building blocks which are added to an amino acid or peptide residue attached to the solid support at the acyl (acid)

30

end of the molecule. After the peptide bond has been formed, the protected group is removed and the cycle repeated. When a peptide having the desired sequence has been synthesized, it is then removed from the support.

5 The second method, the inverse Merrifield method, employs reagents attached to solid supports in a series of columns. The amino acid or peptide residue is passed through these columns in a series to form the desired amino acid sequence.

10 These methods are well known in the art as discussed in U.S. Patent Nos. 4,108,846, 3,839,396, 3,835,175, 4,508,657, 4,623,484, 4,575,541, 4,581,167, 4,394,519 as well as in Advances in Enzymology, 32, 221 (1969) and in PEPTIDES, Vol. 2, edited by Erhard Gross and Johannes Meienhoffer, Academic Press, New York pp. 3-255 (1980) and the contents thereof are incorporated herein by reference as if fully set forth herein.

15 In solid phase polypeptide synthesis, an insoluble solid support or matrix, advantageously in bead form, is used. Such solid supports can be any of the solid phase polymeric substrates conventionally employed for the synthesis of polypeptides. Typical of such polymeric resins are crosslinked polystyrene resins, glass beads, clays, Celite, crosslinked dextran, polyacrylamides, polyamide resins, polyethylene glycol grafted polystyrene, and similar insoluble solid supports which either naturally contain reactive sites for coupling with the amino acid components or which can be provided with such reactive sites. Suitable resins are phenyl ester-type resins or resins with oxime or thioester linkages.

20 Several preliminary operations are necessary before the solid phase synthesis of a peptide can be started. First the supporting resin containing the C-terminal amino acid component of the proposed peptide chain must be prepared. This can be accomplished by any of a number of procedures known to one skilled in the art. Many of these solid supports, derivatized with N-protected amino acids, are commercially available and may be purchased as desired. Many of the common resin linkages (for the preparation of C-terminal peptide amides, peptide acids, and the like) can be prepared with Boc-amino acids as easily as with the other N-protected amino acids, and this may be accomplished by any of a number of procedures known to be skilled in the art.

25 The remaining synthesis to form the desired polypeptide sequence is carried out in the following manner. Before coupling of the second amino acid can take place, the first residue already on the support must be deprotected. Deprotection of the first amino acid residue on the resin as well as of each of the subsequently coupled amino acid residues can be carried out by contacting the protected amino acid residue with an appropriate

deprotecting agent. The deprotecting agents employed for this purpose are well known to those of ordinary skill in the art of peptide synthesis and the particular deprotecting agent employed in any given instance will depend, of course, upon the protecting group used. For example, if the protecting group is t-butyloxycarbonyl, trifluoroacetic acid (usually 50% or higher) in dichloromethane or hydrochloric acid in a suitable solvent such as dioxane may be used for deprotection. On the other hand, if the protecting group is 9-fluorenylmethyloxycarbonyl, basic conditions such as piperidine (usually 20%) in DMF will be the preferred method of deprotection. If the protecting group for the first amino acid attached to the resin is Boc, the deprotecting agent of choice will be 0.5% TFA in dichloromethane. Solutions of glacial acetic acid in trifluoroethanol, ethanol or dichloromethane can also be employed.

After the deprotecting step, the resin is washed with a suitable solvent in order to remove excess deprotecting agents. If the deprotecting agent is a solution of acid, the subsequent step of neutralization is typically carried out with an appropriate non-nucleophilic tertiary amine base or tertiary ammonium trifluoroacetate. Any excess tertiary amine or tertiary ammonium trifluoroacetate salt can be removed with a suitable solvent such as dichloromethane, dimethylformamide, or with solids supports with suitable swelling properties, ethanol or methanol. The resin-bound free amine, thus prepared, is now ready for coupling with the next N-protected amino acid.

Before each coupling step, the N-protected amino acid component to be coupled can be activated, that is, the carboxylic acid can be converted it into a reactive form by any of a number of accepted procedures known to those of ordinary skill in the art of peptide synthesis. In general, an excess of the activated N-protected amino acid component is employed in the reaction.

After the coupling of the second protected amino acid component to the first amino acid component, the attached protected dipeptide is then deprotected, neutralized if necessary, and washed as described above before coupling of the next amino acid derivative is effected. This procedure is repeated until the desired sequence of amino acids has been assembled on the insoluble support. The completed peptide can be removed from the insoluble support by any of the standard methods as, for instance, by cleavage with trifluoroacetic acid (for appropriately functionalized alkoxybenzyl alcohol, alkoxybenzyl amine, or alkoxybenzhydrylamine resins), Pd₀ /tributyltin hydride mixtures in dichloromethane (for appropriately functionalized allyl-type linkers), aminolysis,

alcoholysis, or hydrolysis (for appropriately functionalized of the phenyl ester or oxime type).

After cleavage from the solid support, the resulting peptide requires minimal or no further purification. Because of the very low contamination of byproducts overall yields are found to be high and whatever purification is necessary can be carried out with relative ease. Such purifications are preferably carried out by partition chromatography, ion exchange chromatography, reversed-phase high performance liquid chromatography or a combination of both. Such procedures are well-known to one skilled in the art of peptide synthesis.

The present invention can readily be utilized in liquid phase peptide synthesis. Liquid phase peptide synthesis is based on the method, in which organic solvent soluble polymers are used as a support for synthetic reactions. When the reactions are complete, non-polar solvent (usually, ether) is added to the solution, causing the precipitation of the polymer, which is then isolated by filtration. This precipitation/crystallization allows for removal of reagents and solvents by filtration, thus combining the advantages solution phase chemistry and the utility of solid phase purification (D. J. Gravert, Kim D. Janda. Organic Synthesis on Soluble Polymer Supports: Liquid Phase Methodologies. *Chem. Rev.* 1997, v.97, p. 489-509).

Compounds of the formula I can be prepared by any method known to a person skilled in the art.

The following examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

25

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1

30 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBt or Dhbt-OH).

a) 2-Aminobenzhydrolamic acid.

To a cooled solution of 24 g of sodium hydroxide in 150 ml of water was added slowly with stirring 20.8 g of hydroxylamine hydrochloride. To this solution 22.6 g of

5 methyl anthranilate was added, following by methyl alcohol in amount to bring it into solution. This solution was allowed to stir for three days at room temperature. Solution was distilled under reduced pressure until the sodium salt of the hydroxamic acid was precipitated, leaving about 50-70 ml of the mother liquid in the flask. The salt was filtered off and washed with ether. The filtrate was made acid with hydrochloric acid and the free hydroxamic acid was precipitated. The crude product was washed with ether, giving the desired product with 65% yield. After recovering from mother liquid the yield can be increased up to 80%. Recrystallization of ether using a Soxhlet extractor gave light yellow to brown crystals with melting point 146-150°C.

10 Anal. Calcd. for C₇H₈N₂O₂: C, 55.26, H, 5.26, N, 18.46

Found: C, 55.06, H, 5.22, N, 18.20

15 b) 14.5 g of 2-aminobenzhydroxamic acid in 25 ml of hydrochloric acid (conc.) was diluted with 270 ml of cold water and then was diazotized with 7.2 g of sodium nitrite in 25 ml of water at 0-5 °C. The resulted solution was stirred at room temperature for 1-2 h and filtered to afford 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine with the yield 75-82%. The product was crystallized from ethanol, giving white crystals with m.p. 180-183 °C. ¹H NMR (DMSO-d₆): δ = 2.55 (s, OH), 7.99 (dt), 8.14 (dt), 8.31 (d), 8.41 (dd). UV: λ_{max} = 302 nm. IR: ν = 1630, 1670 cm⁻¹ (C=O, N=N).

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EXAMPLE 2

7-Chloro-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine.

25 Methyl 4-chloroanthranilate is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 3

3,4-Dihydro-3-hydroxy-7-methyl-4-oxo-1,2,3-benzotriazine.

30 Methyl 4-methylantranilate is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 43,4-Dihydro-3-hydroxy-6,7-dimethyl-4-oxo-1,2,3-benzotriazine.

5 Methyl 4,5-dimethylanthranilate is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 510 3,4-Dihydro-3-hydroxy-8-trifluoromethyl-4-oxo-1,2,3-benzotriazine.

Methyl 2-amino-3(trifluoromethyl)benzoate is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

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EXAMPLE 66-Chloro-3,4-Dihydro-3-hydroxy-7-nitro-4-oxo-1,2,3-benzotriazine.

20 5-Chloro-4-nitroanthranilic acid (US 4959367) is converted to its methyl ester, following by reaction with hydroxylamine hydrochloride and the product is diazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 73,4-Dihydro-3-hydroxy-6-fluoro-4-oxo-1,2,3-benzotriazine.

25 Methyl 5-fluoroanthranilate is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 8

30

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,8-tetraazanaphthalene.

Methyl ester of 2-aminonicotinic acid is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

5

EXAMPLE 9

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3 -triazaanthracene.

Methyl ester of 3-amino-2-naphthoic acid is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

EXAMPLE 10

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,4,8-pentaazanaphthalene.

Methyl ester of 3-aminopyrazine-2-carboxylic acid is reacted with hydroxylamine hydrochloride and the product is deazotized in accordance with the procedure of Example 1 to yield the above-identified compound.

All synthesized derivatives (examples 1-10) have acceptable analytical characteristics: ^1H NMR spectra correspond to structure, UV: $\lambda_{\text{max}} = 300\text{-}305$ nm. IR: $\nu = 20$ 1630, 1670 cm^{-1} (C=O, N=N).

EXAMPLE 11

3-(Diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one

To a mixture of 71 g (0.4 mol) of 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and 44.5 g of triethylamine in 400 ml of methylene chloride, 86 g (0.5 mol) of diethyl chlorophosphate in 200 ml of methylene chloride was added dropwise with stirring at 0-5°C. The mixture was stirred for 3 h at room temperature under TLC control. After finishing the reaction, triethylamine hydrochloride was filtered off and solvent was evaporated in vacuum. The residue was dissolved in 200 ml of ethyl acetate, washed with water and brine, then dried over magnesium sulfate. The solution was concentrated in vacuum to volume of 30-40 ml and hexanes (fraction C₆) or petroleum ether were added causing the precipitation of title compound. This compound was filtered off, dried and recrystallized from ethyl

acetate/hexane, giving 3-(diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one with m.p. 72-75 °C. ^1H NMR (CDCl_3): δ = 1.45 (t, 6H, CH_3), 4.50 (m, 4H, CH_2), 7.99 (dt, 1H_{ar.}), 8.14 (dt, 1H_{ar.}), 8.31 (d, 1H_{ar.}), 8.41 (dd, 1H_{ar.}). ^{31}P NMR (CDCl_3): δ -30 ppm. UV: λ_{max} = 302 nm. IR: ν = 1710, 1300 cm^{-1} (C=O, P=O).

5

EXAMPLE 12

3-(Diethoxyphosphoryloxy)-1,2,3,8-tetraazanaphthalene -4(3H)-one

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,4,8-pentaazanaphthalene (from example 10) is reacted with diethyl chlorophosphate in accordance with the procedure of Example 11 to yield the above-identified compound.

EXAMPLE 13

15 7-Chloro-3-(diethoxyphosphoryloxy)-1,2,3-benzotriazine -4(3H)-one

7-Chloro-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (from example 2) is reacted with diethyl chlorophosphate in accordance with the procedure of Example 11 to yield the above-identified compound.

All synthesized derivatives (from examples 12 and 13) have acceptable analytical characteristics: ^1H NMR spectra correspond to structure, UV: λ_{max} = 300-305 nm. IR: ν = 1710-1720, 1300-1310 cm^{-1} (C=O, P=O).

EXAMPLE 14

25 O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

To a 99 g (0.306 mol) of chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate in 300 ml of acetonitrile was added 50 g (0.306 mol) of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (from example 1) and 37.1 g (0.367 mol) of triethylamine in 300 ml of acetonitrile with stirring at 0-5°C. After stirring at room temperature for 3 h, the solid was filtered off, washed twice with 30 ml of acetonitrile. The solvent was evaporated to volume of 250-270 ml and 300 ml of isopropyl alcohol was added with stirring. Filtration of solid, washing with 50 ml of isopropyl alcohol and dried in

vacuum gave 68 g of desired compound and additional 32 g was received from mother liquid (80% overall yield), m.p. 138-140°C. ^1H NMR (CD₃CN, ppm): δ = 3.04 (s, 6H, CH₃), 3.40 (s, 6H, CH₃), 8.00 (dt, 1H_{ar.}), 8.14 (dt, 1H_{ar.}), 8.31 (d, 1H_{ar.}), 8.41 (dd, 1H_{ar.}). ^{31}P NMR (CD₃CN, ppm): δ -117 (hept, PF₆). ^{19}F NMR (CD₃CN, ppm): δ -72.2 (d, PF₆).

5 Alternatively, halo-N,N,N',N'-tetramethylformamidinium hexafluoro- phosphate wherein halo is fluoro or bromo may be reacted with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form the above-identified compound.

EXAMPLE 15

10

O-(3,4-Dihydro-4-oxo-1,2,3,4,8-pentaazanaphthalen-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

15 By reaction of halo-N,N,N',N'-tetramethylformamidinium hexafluoro- phosphate (wherein halo is fluoro, chloro or bromo) with 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,4,8-pentaazanaphthalene (from example 10) in accordance with the procedure of Example 14, the title compound is prepared.

EXAMPLE 16

20

O-(3,4-Dihydro-6-fluoro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

25 By reaction of halo-N,N,N',N'-tetramethylformamidinium hexafluoro- phosphate (wherein halo is fluoro, chloro or bromo) with 3,4-dihydro-3-hydroxy-6-fluoro-4-oxo-1,2,3-benzotriazine (from example 7) in accordance with the procedure of Example 14, the title compound is prepared.

EXAMPLE 17

30

O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-bis(tetramethylene)uronium hexafluorophosphate.

By reaction of halo-N,N,N',N'-bis(tetramethylene)formamidinium hexafluorophosphate (wherein halo is fluoro, chloro or bromo) with 3,4-dihydro-3-hydroxy-

4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 14, the title compound is prepared.

EXAMPLE 18

5

O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-bis(pentamethylene)uronium hexafluorophosphate.

10 By reaction of halo-N,N,N',N'-bis(pentamethylene)formamidinium hexafluorophosphate (wherein halo is fluoro, chloro or bromo) with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 14, the title compound is prepared.

EXAMPLE 19

15 O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'
dimethyleneuronium hexafluorophosphate.

a). 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate.

14.8 g (0.05 mol) of triphosgene in 30 ml of methylene chloride was added dropwise to a solution of 1,3-dimethylimidazolidinone (20.7 g, 0.16 mol) in 50 ml of methylene chloride with stirring at 0-5°C. The mixture was stirred at room temperature for 5 h. 30 g (0.18 mol) of sodium hexafluorophosphate in water was added to this solution and stirred for 3 h. The organic layer was separated, washed twice with water, dried over magnesium sulfate and evaporated to give a white solid, yield 37.6 g (90%).

25 Alternatively, phosphorus oxychloride, or diphosgene, or phosgene, or oxalyl chloride could be substituted for the triphosgene in the above procedure to afford the above-identified compound.

b). 2-Fluoro-1,3-dimethylimidazolidinium hexafluorophosphate.

30 A mixture of 2-chloro-1,3-dimethylimidazolidinium chloride (8.8 g, 0.052 mol), prepared by evaporation of reaction mixture after interaction of 1,3-dimethylimidazolidinone with triphosgene and potassium fluoride (dried at 125°C overnight) (3 g, 0.052 mol) in 25 ml of acetonitrile was stirred at room temperature for 24 h. The mixture was filtered and concentrated in vacuum. The crude product was redissolved in acetonitrile, precipitated with ether, filtered and dried to give 10.6 g (78%) of title compound, m.p. 158-160 °C.

c). By reaction of 2-halo-1,3-dimethylimidazolidinium hexafluoro- phosphate (wherein halo is fluoro, chloro or bromo) with 3,4-dihydro-3-hydroxy- 4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 14, the title compound is prepared.

5

EXAMPLE 20

O-(5-Chloro-3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'-trimethyleneuronium hexafluorophosphate.

10 By reaction of 2-halo-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium hexafluorophosphate (wherein halo is fluoro, chloro or bromo) with 5-chloro-3,4-dihydro-3-hydroxy- 4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 14, the title compound is prepared.

15 All synthesized derivatives (from examples 15-20) have acceptable analytical characteristics: ^1H , ^{31}P , ^{19}F NMR spectra correspond to structure.

EXAMPLE 21

20 O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate.

Chloro-N,N,N',N'-tetramethylformamidinium tetrafluoroborate is reacted with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and triethylamine in accordance with the procedure of Example 14 to yield titled compound, m.p. 142-147°C (dec.). ^1H NMR (CD_3CN , ppm): δ = 3.04 (s, 6H, CH_3), 3.40 (s, 6H, CH_3), 8.00 (dt, 1H_{ar.}), 8.14 (dt, 1H_{ar.}), 8.31 (d, 1H_{ar.}), 8.41 (dd, 1H_{ar.}). ^{19}F NMR (CD_3CN , ppm): δ -151.6 (BF_4^-).

EXAMPLE 22

30 O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'-dimethyleneuronium tetrafluoroborate.

2-Chloro-1,3-dimethylimidazolidinium tetrafluoroborate was prepared according to Example 19, using sodium tetrafluoroborate instead of sodium tetrafluorophosphate. By

reaction of 2-chloro-1,3-dimethylimidazolidinium tetrafluoroborate with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 14, the title compound is prepared.

Alternatively, 2-halo-1,3-dimethylimidazolidinium tetrafluoroborate wherein halo is 5 fluoro or bromo may be reacted with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form the above-identified compound.

EXAMPLE 23

10 [(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-tris(dimethylamino)- phosphonium hexafluorophosphate.

a). Chlorotris(dimethylamino)phosphonium hexafluorophosphate

32.2 g (0.21 mol) of phosphorus oxychloride was added to 50 ml of dry methylene chloride. To this mixture 35.8 g (0.2 mol) of hexamethylphosphoramide (HMPA) in 100 ml 15 of methylene chloride was added dropwise with stirring, keeping the temperature $-5\pm2^{\circ}\text{C}$. After addition of HMPA, the mixture was stirred at 0°C , for 1 h, then cooled to -5°C and 21.8 g (0.215 mol) of triethylamine was added dropwise, keeping the temperature $<0^{\circ}\text{C}$. After 20 addition of triethylamine, the mixture was stirred for 0.5 h and poured to the precooled ($4\text{--}5^{\circ}\text{C}$) mixture of water (500 ml) and 30.6 g of triethylamine. The organic layer was separated and to water solution was added 36.8 g of potassium hexafluorophosphate in 450 ml of 25 water, inducing the precipitation of chlorotris(dimethylamino)phosphonium hexafluorophosphate. The solid was filtered, washed with cold water and dried. The yield is 54.5 g (80%), m.p. $>300^{\circ}\text{C}$ (dec.). ^1H NMR (CDCl_3 , ppm): $\delta = 2.80$ and 2.83 (ds, 18H, CH_3). ^{31}P NMR (CDCl_3 , ppm): $\delta = 142$ (hept, PF_6), 55 (s, Me_2NPOCl). ^{19}F NMR (CDCl_3 , ppm): $\delta = 70.2$ (d, PF_6).

b). To 17.2 g (0.05 mol) of chlorotris(dimethylamino)phosphonium hexafluorophosphate and 8.15 g (0.05 mol) of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in 100 ml of methylene chloride was added 5.6 g (0.055 mol) of triethylamine in 10 ml of methylene chloride. The mixture was stirred for 0.5 h at room temperature and 30 150 ml of ether was added. The precipitate was collected on a filter, washed with ether and dried in vacuum. The yield 42.35 g (90%), m.p. $140\text{--}142^{\circ}\text{C}$. ^1H NMR (CDCl_3 , ppm): $\delta = 2.95$ and 2.97 (ds, 18H, CH_3), 8.01 (dt, 1H_{ar.}), 8.15 (dt, 1H_{ar.}), 8.32 (d, 1H_{ar.}), 8.43 (dd, 1H_{ar.}). ^{31}P NMR (CDCl_3 , ppm): $\delta = 143.9$ (hept, PF_6), 43.8 (s, Me_2NPO).

Alternatively, bromo-tris(dimethylamino)-phosphoniumhexafluorophosphate may be reacted with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form the above-identified compound.

Alternatively, titled compound can be prepared by reaction of equimolar amounts of tris(dimethylamino)phosphine, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and triethylamine in carbon tetrachloride/THF mixture at -30 to -25°C, following by addition of potassium hexafluorophosphate in water.

Alternatively, hexachloroethane could be substituted for carbon tetrachloride in the above procedure to afford the title compound.

10

EXAMPLE 24

[(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-tris(pyrrolidino)- phosphonium hexafluorophosphate.

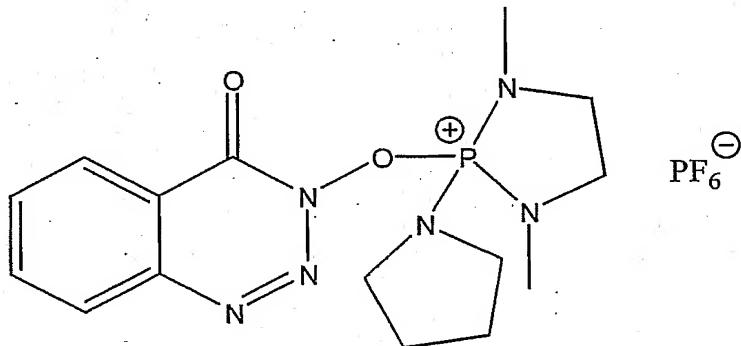
By reacting halotris(pyrrolidino)phosphonium hexafluorophosphate (wherein halo is chloro or bromo) with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 23, the title compound is prepared.

Alternatively, titled compound can be prepared by reaction of equimolar amounts of tris(pyrrolidino)phosphine, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and triethylamine in carbon tetrachloride/THF mixture at -30 to -25°C, following by addition of potassium hexafluorophosphate in water.

Alternatively, hexachloroethane could be substituted for carbon tetrachloride in the above procedure to afford the title compound.

EXAMPLE 25

2-[(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-1,1-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate.



5

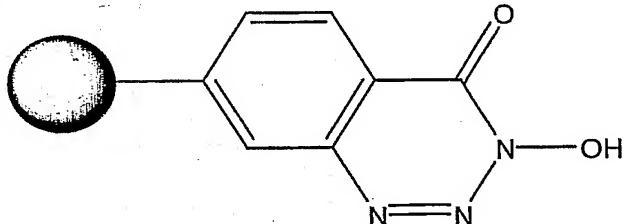
By reacting 2-halo-1,1-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (wherein halo is chloro or bromo) with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in accordance with the procedure of Example 23, the title compound is prepared.

10 Alternatively, titled compound can be prepared by reaction of equimolar amounts of 2-N-pyrrolidino-1,3-dimethyl-1,3,2-diazaphospholane, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and triethylamine in carbon tetrachloride/THF mixture at -30 to -25°C, following by addition of potassium hexafluorophosphate in water.

15 Alternatively, hexachloroethane could be substituted for carbon tetrachloride in the above procedure to afford the title compound.

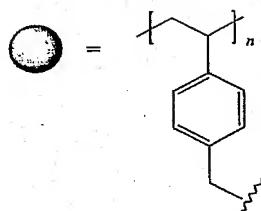
EXAMPLE 26

Polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine



20

where



Polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine was prepared from polystyrene-2% divinylbenzene copolymer resin (200-400 mesh) according to the reported method for polymer-bound HOBr (Eur. J. Biochem., 1975, v. 59, p. 55). The activity of the 5 prepared polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine was determined, according to method (Tetrahedron Lett., 1998, v.39, p.1321) to be 0.27-0.28 mmol/g.

EXAMPLE 27

10 Polymer-bound O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

Polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (from example 28) and 3 equivalent of chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate and 3.2 equivalent of triethylamine in acetonitrile was stirred at room temperature until total disappearance of the broad O-H bond at 3450-3500 cm⁻¹ in IR spectrum (~24 h). The 15 resulting polymer was filtered off, washed with methanol, acetonitrile and, finally, with ether, then dried at 50-60 °C in vacuum. The obtained resin showed a strong band at 1670 cm⁻¹ in IR spectrum, which could be assigned to C=N group.

Alternatively, halo-N,N,N',N'-tetramethylformamidinium hexafluoro- phosphate wherein halo is fluoro or bromo may be reacted with polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form the above-identified compound. 20

Alternatively, diethyl chlorophosphate may be reacted with polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form polymer-bound 3-(diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one.

Alternatively, polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine may 25 be reacted with halo derivatives and their hexafluorophosphates and tetrafluoroborates mentioned in examples 14-25 to formed polymer-bound derivatives.

EXAMPLE 28

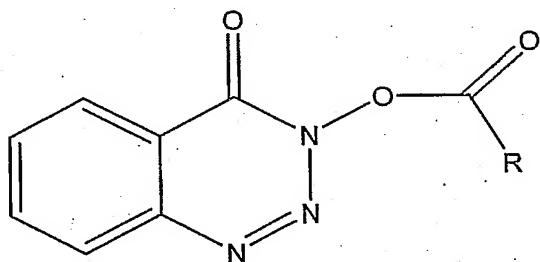
Polymer-bound O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate.

5 Polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (from example 28) and 3 equivalent of chloro-N,N,N',N'-tetramethylformamidinium tetrafluoroborate and 3.2 equivalent of triethylamine in acetonitrile was stirred at room temperature until total disappearance of the broad O-H bond at 3450-3500 cm⁻¹ in IR spectrum (~24 h). The resulting polymer was filtered off, washed with methanol, acetonitrile and, finally, with 10 ether, then dried at 50-60 °C in vacuum. The obtained resin showed a strong band at 1670 cm-1 in IR spectrum, which could be assigned to C=N group.

15 Alternatively, halo-N,N,N',N'-tetramethylformamidinium tetrafluoro- borate wherein halo is fluoro or bromo may be reacted with polymer-bound 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine to form the above-identified compound.

EXAMPLE 29

Preparation of active O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-derived esters.



20

for example R = Alkyl, aryl, FmocNHCH(R'), CbzNHCH(R'), AcNHCH(R')

1) From acyl halogenides.

25

a) Preparation of acyl chlorides.

The Fmoc amino acid (1 mmol) was dissolved or suspended in 10 ml of methylene chloride and after the addition of freshly distilled thionyl chloride (10 mmol) the mixture was refluxed for 0.5 h. Reevaporation in vacuum with methylene chloride 2-3 times gave an oil

or a solid free from excess of thionyl chloride, which can be purified by precipitation from methylene chloride solution with hexane. For example, by this procedure the following Fmoc-amino acid chlorides were prepared: Fmoc-Gly-Cl, Fmoc-Ala-Cl, Fmoc-Leu-Cl, Fmoc-Pro-Cl, Fmoc-Val-Cl, Fmoc-Ser(Bzl)-Cl, Fmoc-Phe-Cl.

5 Alternatively, carboxylic acids with protected amino, hydroxy or thio groups could be substituted for the Fmoc amino acid in the above procedure to afford acyl chlorides.

Alternatively, protected peptides can be converted into protected peptide acid chlorides, according to above procedure;

10 A suspension of Fmoc-peptide acid (1 mmol) in 10 ml of methylene chloride was treated with thionyl chloride (1.5 mmol) and the mixture was stirred for 24 hr at room temperature under nitrogen. Evaporation in vacuum, followed by the addition of methylene chloride and reevaporation gave a thionyl chloride free solid. The obtained solid was dissolved in methylene chloride and precipitate by addition of hexane. The resulting solid was filtered and dried, for example: Fmoc-Leu-Ala-Cl (80% yield, m.p. 66-67°C [lit. 65-68°C [V.V.Suresh Babu, K.Gayathri, H.N. Gopi. *Synth. Commun.*, 1999, v. 29, p. 79]; Fmoc-Tyr(Bzl)-Pro-Cl (65% yield, m.p. 95-96°C).

20 b). To solution of acyl chloride (1 mmol) in 10 ml of methylene chloride was added 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (1 mmol) and diisopropylethylamine (1.1 mmol) at -10°C. The mixture was stirred for 3 h at room temperature. The solids was filtered off, washed with 3 ml of methylene chloride and combined filtrates was evaporated in vacuum, giving solid, which was purified by crystallization or precipitation from methylene chloride with ether or hexane.

25 For amino acid chlorides the use of organic base leads to formation of oxazolone as a main reaction product. Because of this the synthesis of amino acid active esters was performed with sodium or potassium salts of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine instead of amine.

30 To solution or suspension of Fmoc amino acid chloride (1 mmol) in 10 ml of THF was added potassium salt of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (1 mmol) at -5°C. The mixture was stirred for 5 h at room temperature. The solids was filtered off, washed with 3 ml of THF and combined filtrates was evaporated in vacuum, giving solid, which was purified by crystallization or precipitation.

Alternatively, each of presented 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives may be reacted with acyl chloride to form esters.

Alternatively, instead of acyl chloride can be used acyl fluoride prepared according to J. Prakt.Chem., 2000, v.342, p.711.

5

2). Using carbodiimides

To solution of Fmoc amino acid (10 mmol) in 30 ml of THF was added DCC(10 mmol) in 20 ml of THF with stirring at -10°C for 10-15 min. Then solution of 10 mmol of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine in 20 ml of THF was added and the mixture was stirred at that temperature for 0.5 h, then at 0-4°C for 5 h. The solids were filtered off, solvent evaporated in vacuum and residue was purified by crystallization or precipitation.

3). Using Boc-anhydride.

15 To a solution of Boc₂O (1.2 eq.) in 20 ml of acetonitrile was added Fmoc amino acid, followed by 1 eq. of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine and 0.3-0.5 eq. of dimethylaminopyridine (DMAP). The mixture was stirred at room temperature for 2-3 h. The solvent was evaporated in vacuum, the residue was dissolved in ethylacetate, or ether or methylene chloride and washed with diluted hydrochloric acid, water, 10% solution of sodium bicarbonate, water, then dried over sodium sulfate and evaporated in vacuum. The solid was purified by crystallization or precipitation.

4). Using bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate.

25 A solution of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (0.1 mol) in 200 ml of THF was vigorously stirred with a mechanical stirrer, whereupon 0.2 mol of oxalyl chloride was slowly added at -5-0°C. After stirring for 3 h at room temperature, a precipitate was filtered, washed with THF and dried in vacuum. The compound is pure enough to be used in further preparation of active ester. Suspended bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate (1 mmol) in 10 ml of THF was added to a solution of organic acid (1 mmol) and pyridine (1 mmol) in 10 ml of THF. The reaction mixture became a clear solution. After the reaction mixture was stirred for 1-2 h at room temperature, the mixture was worked up as described in above example 29/3.

EXAMPLE 30Preparation of carboxylic acid esters using 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives

5 Suspended bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate (1 mmol) in 10 ml of THF was added to a solution of organic acid (1 mmol) and pyridine (1 mmol) in 10 ml of THF. The reaction mixture became a clear solution. After the reaction mixture was stirred for 1-2 h at room temperature, a solution of alcohol (1.1 mmol) and DMAP (1.1 mmol) in 5 ml of THF was added at room temperature. Stirring was continued for an additional 5-6 h. The 10 reaction mixture was quenched with water and the product was extracted with ethyl acetate, then the ethyl acetate solution was washed with 5% bicarbonate solution, 1N HCl solution, water, brine and dried over sodium sulfate. After removal of the solvent in vacuum, the residue was purified by distillation or crystallization, affording the esters with 92-98% yield.

15 Methyl trans-cinnamate (97%), m.p. 34-35°C; ^1H NMR (CDCl_3): δ = 3.80 (s, 3H), 6.44 (d, 1H), 7.38 (m, 3H), 7.52 (m, 2H), 7.70 (d, 1H).

Ethyl diphenylacetate (95%), m.p. 56-58°C; ^1H NMR (CDCl_3): δ = 1.26 (t, 3H), 4.20 (q, 2H), 5.0 (s, 3H), 7.52 (s, 1H), 7.30 (m, 10 H).

20 Menthyl benzoate (92%), m.p. 53-54.58°C; ^1H NMR (CDCl_3): δ = 0.80 (d, 2H), 0.90 (d, 3H), 0.94 (d, 3H), 1.14 (m, 3H), 1.58 (m, 2 H), 1.74 (m, 2H), 1.96 and 1.98 (d sept., 1H), 2.14(m, 1H), 4.94 and 4.96 (dt, 1H), 7.44 (m, 2H), 7.54 (m, 2H), 8.08 (m, 2H).

25 5'-O-(4,4'-Dimethoxytrityl)-3'-O-[N-(9-fluorenylmethyoxycarbonyl)glycyl]thymidine (92% after flash chromatography on silica gel, using gradient 0-1% methanol in chloroform); ^1H NMR (CDCl_3): δ = 1.40 (s, 3H), 2.32 (s, 2H), 3.48 (s, 2H), 3.78 (s, 6H), 4.0 (d, 2 H), 4.14 (s, 1H), 4.22 (t, 1H), 4.42(d, 2H), 5.50 (s, 2H), 6.44 (t, 1H), 6.84 (d, 4H), 7.30 (m, 13H), 7.60 (d, 3H), 7.74 (d, 2H), 9.42 (s, 1H).

Alternatively, active O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-derived esters could be substituted for the bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate in the above procedure to afford the esters of carboxylic acids.

EXAMPLE 31Preparation of thioesters using 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives

5 Suspended bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate (1 mmol) in 10 ml of THF was added to a solution of organic acid (1 mmol) and pyridine (1 mmol) in 10 ml of THF. The reaction mixture became a clear solution. After the reaction mixture was stirred for 1-2 h at room temperature, a solution of thiol (1.1 mmol) and triethylamine (1.1 mmol) in 5 ml of THF was added at room temperature. Stirring was continued for an additional 5-6 h.

10 The reaction mixture was quenched with water and the product was extracted with ethyl acetate, then the ethyl acetate solution was washed with 5% bicarbonate solution, 1N HCl solution, water, brine and dried over sodium sulfate. After removal of the solvent in vacuum, the residue was purified by distillation or crystallization, affording thioesters with 90-98% yield.

15 Ethyl thiobenzoate (96%), oil; ^1H NMR (CDCl_3): δ = 1.38 (t, 3H), 3.10 (q, 2H), 7.644 (m, 5H).

20 Alternatively, active O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-derived esters could be substituted for the bis(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl) oxalate in the above procedure to afford the esters of carboxylic acids.

EXAMPLE 32Preparation of amides using 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives25 1.) Using active esters

30 To 1 mmol of 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl carboxylic acid ester in 10 ml of acetonitrile was added 1 mmol of amine. The reaction was stirred at room temperature under TLC control. After complete disappearance of starting ester, the solvent was evaporated and residue was purified by crystallization, precipitation or distillation, affording amide with 85-97% yield.

35 N-Phenethylcinnamide (96%), m.p. 126-128°C; ^1H NMR (CDCl_3): δ = 2.90 (dt, 2H), 3.68 (dt, 2H), 5.70 (s, 1H), 6.34 (d, 1H), 7.30 (m, 8 H), 7.62 (d, 1H).

N-Cyclohexylbenzamide (93%), m.p. 144-147°C; ^1H NMR (CDCl_3): δ = 1.20 (m, 3H), 1.43 (m, 2H), 1.70 (m, 3H), 2.0 (m, 2H), 4.0 (m, 1H), 6.02 (s, 1H), 7.44 (m, 3H), 7.75 (m, 2H).

5 Boc-L-Leucine N-methyl-O-methylcarboxamide, colorless syrup, IR (liquid film) cm^{-1} : 2960(s), 1714(s), 1665(s); ^1H NMR (CDCl_3) δ : 0.92 [t, 6H, J = 6.8, $\text{CH}(\text{CH}_3)_2$], 1.40, [s, 9H, $\text{C}(\text{CH}_3)_3$], 1.38-1.44 (m, 2H, $\text{C}_3\text{-H}$), 1.59-1.76 (m, 1H, $\text{C}_4\text{-H}$), 3.17, and 3.14 (s and a rotamer singlet, 3H, N-CH_3), 3.76 and 3.67 (s, and a rotamer singlet, 3H, O-CH_3), 4.7 (m, 1H, $\text{C}_2\text{-H}$), 5.06 (m, 1H, N-H).

10 Alternatively, active esters can be prepared in situ, using procedures 1-4 from Example 29 without separation and purification of active esters. In such cases amine was added to reaction mixture and then the reaction should be followed the procedure 32/1.

2). Using O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

15 To a mixture of the carboxylic acid (1 mmol), the corresponding amine (1 mmol) and the organic base (1.5-2 mmol) in the appropriate solvent (for example, triethylamine or pyridine in acetonitrile or diisopropylamine in DMF, depending on solubility of acid and amine in these solvents) was added O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (1 mmol) with stirring at room 20 temperature. The solution was stirred until completion (TLC control) and brine was added. The mixture was extracted with ethyl acetate, washed with 1N HCl, 10% sodium bicarbonate, water and dried over sodium sulfate. Evaporation afforded crude product, which was purified by crystallization or distillation.

25 N-Cholyl L-valine methyl ester, white solid after flash chromatography on silica gel, using methylene chloride-methanol (95:5) as eluent, ^1H NMR (CDCl_3) δ : 0.68 (s, 3H), 0.88 (s, 3H), 0.93 (d, 2H), 1.00 (d, 3H), 1.03-2.26 (m, 25H), 3.48 (m, 1H), 3.74 (s, 3H), 3.84 (s, 1H), 3.98 (s, 1H), 4.55 (dd, 1H), 6.0 (d, 1H).

30 N-Dihydrocinnamoyl-S-trityl-L-cysteine methyl ester, white foam after flash chromatography on silica gel, using ethyl acetate-hexanes as eluent, ^1H NMR (CDCl_3) δ : 2.45 (t, 2H), 2.63 (d, 2H), 2.94 (m, 2H), 3.68 (s, 3H), 4.58 (dt, 1H), 5.80 (d, 1H), 7.26 (m, 20H).

Fmoc-L-Alanine N-methyl-O-methylcarboxamide (92%), m.p. 123-124°C.

Fmoc-L-Alanine N-benzyl-O-benzylcarboxamide (90%), m.p. 146-147°C.

Alternatively, compounds, prepared according to procedures of Examples 15-28 could be substituted for the O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate in the above procedure to afford the above-identified amides.

5

EXAMPLE 33

Preparation of peptides using 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives

10 1. Preparation of peptides, using carbodiimide/3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine derivatives.

General procedure for test coupling.

15 0.105 mmol of a protected amino acid, 0.1 mol of amino acid ester or amide, 0.11 mmol of additive (HOX, where X = Bt, At, OBT, OBT) were dissolved in 1 ml of DMF or 1.3 ml of trifluoroethanol/chloroform (1:3) or 1.3 mol chloroform/phenol (1:3) and the solution was cooled to 0°C and 0.11 mmol of EDAC or other condensing agent was added. The mixture was stirred at this temperature for 1 h and overnight at room temperature. The mixture was extracted with 25 ml of ethyl acetate and organic solution was washed with 1N HCl, 10% sodium bicarbonate and water, then dried over sodium sulfate. The solvent was removed in vacuum and the residue was directly analyzed by HPLC and NMR. The results are provided in Table 1.

20 Table 1. Comparison of different additives in the model coupling
Z-Phe-Val-OH + H-Pro-NH₂, using EDAC as condensing reagent

25

Additive	Solvent					
	DMF		TFE/CHCl ₃		CHCl ₃ /Phenol	
	yield, %	LDL, %	yield, %	LDL, %	yield, %	LDL, %
HOAt ^a	84.8	4.7	68.8	17.9	88.0	0.5
HOOBt	89.1	7.3	65.8	11.4	83.4	0.2
HOBT	86.7	18.9	68.7	37.8	78.0	0.2
6-Cl-HOOBt ^b	88.0	7.5	66.0	11.6	88.0	0.25

8-N-HOOBt ^c	90.1	5.8	66.2	11.0	88.2	<0.1
5,8-N,N-HOOBt ^d	92.5	4.8	68.9	10.1	89.4	<0.1

a). Data were taken from J. Org. Chem., 1995, v.60, p.3561
5 b). 6-Chloro-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
c). 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,8-tetraazanaphthalene
d). 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,5,8-pentaazanaphthalene

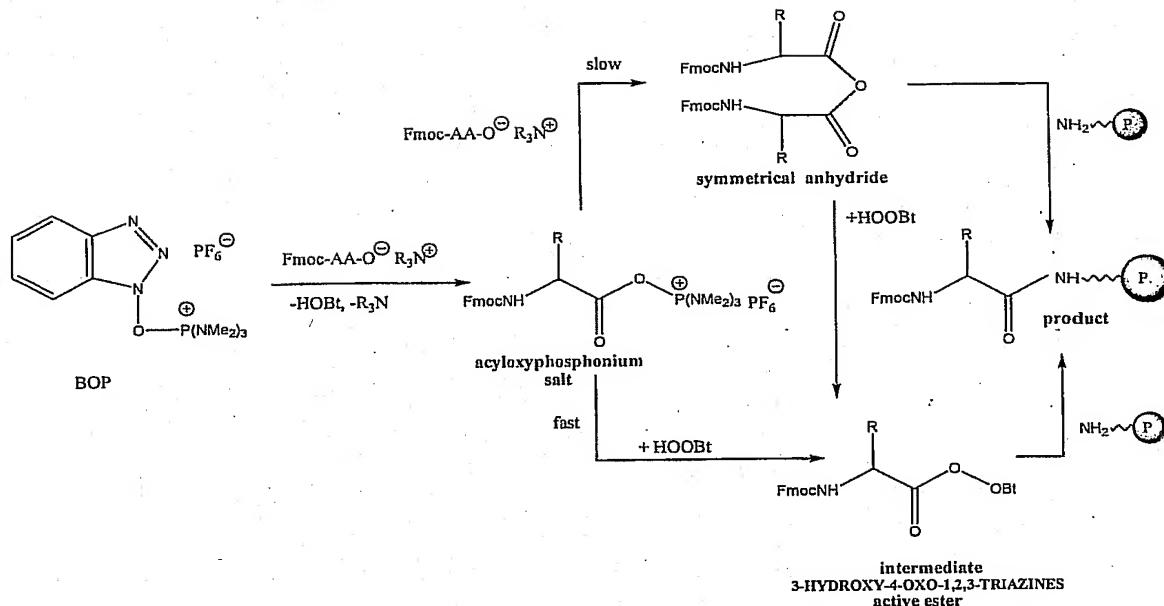
2. Preparation of peptides, using phosphonium coupling reagents (DOP and PyDOP).

10

To a methylene chloride solution, containing 1 eq. of N-protected amino acid, the following compounds were added: 1 eq. of phosphonium coupling reagent, 1.05 eq. of C-protected amino acid hydrochloride and 2.75 eq. of diisopropylethylamine. TLC monitoring showed an immediate reaction. The reaction was stirred for 1-2 h at room temperature, the solvent was evaporated, the residue dissolved in ethyl acetate, washed with 5% KHSO4 (or 1N HCl), brine, 5% sodium bicarbonate, water, dried over sodium sulfate and evaporated in vacuum. All prepared peptides were purified by crystallization, or flash chromatography on silica gel.

20 3. Preparation of peptides, using combination of phosphonium coupling reagents (BOP and PyBOP) with HOOBt (activated coupling).

Without being limited to any particular theory or mechanism, one feasible reaction mechanism for the activated HOOBt coupling, using phosphonium reagents in accordance 25 with the invention is as follows:



Without addition of HOOBt, the initial reaction between BOP and protected amino acid in the presence of tertiary amine (diisopropylethyl amine, N-methylmorpholine, collidine) 5 consists in fast formation of acyloxyphosphonium salt, which is slowly converted to symmetrical anhydride. This anhydride is transformed to hydroxybenzotriazole active ester by HOBt liberated from BOP in the initial step or can directly react with available reactive functionalities (for example amino acids) to form desirable products. In the final step, intermediate benzotriazole active ester reacts with amino group to form the product. 10 Introduction of HOOBt, more active than HOBt, lead to immediate formation of benzotriazine active ester without formation of less reactive symmetrical anhydride. Due to this, the efficiency of coupling is increased and many side reactions, connected with symmetrical anhydride (for example, dehydration of asparagine and glutamine) may be eliminated.

15

Table 2. Coupling efficiency ranking of activation methods for solid phase peptide synthesis.

	Reagent	Relative efficiency
20		
1.	BOP + HOOBt	2.5
2.	PyBOP + HOOBt	2.3

3.	BOP + HOBT	1.7
4.	PyBOP + HOBT	1.4
5.	BOP + 10 min preactivation	1.35
6.	BOP = preformed symmetrical anhydride	1.0
5	7. DCC + HOBT	0.8

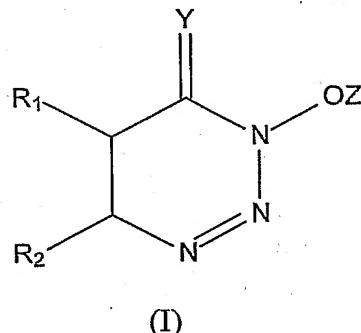
Alternatively, HOOBt and other its derivatives may be used together with other HOBT (HBTU, TBTU), pentafluorophenyl, HONSu (HSTU, TSTU), HOPy (TPTU), HONB (TNTU) – based uronium (aminium) coupling reagents.

Alternatively, HOOBt and other its derivatives may be used together with halogen based coupling reagents (BrOP, TFFH, PyBrOP, Mukayama reagents and other).

It will be appreciated by a person skilled in the art that the present invention is not limited by what has been particularly shown and described hereinabove. Rather, the scope 15 of the invention is defined by the claims which follow:

What is claimed is:

1. A compound represented by the structure of formula (I):



5

wherein

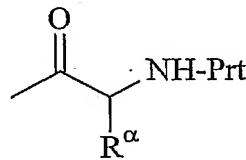
Y is O, S or NR₃ wherein R₃ is hydrogen, alkyl or aryl;

10 R₁ and R₂ are independently of each other selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, halogen, haloalkyl, alkylamino, dialkylamino, arylamino, diarylamino, alkylarylarnino, alkoxy, aryloxy, arylalkyloxy, carboxyalkyl, carboxyaryl, carboxyaryllalkyl, alkylthio, arylthio, arylalkylthio, cyano, nitro, carbonyl, alkylcarbonyl, arylcarbonyl, arylalkylcarbonyl, alkanoyl, sulfonyl, alkylsulfonyl, arylsulfonyl, arylalkylsulfonyl, a polymer-based solid support and P(O)(OR₄)₂ wherein R₄ is hydrogen, alkyl or aryl; or R₁ and R₂ together with the carbon atoms to which they are attached form an aryl, heteroaryl, carbocyclic or heterocyclic ring, wherein said aryl, heteroaryl, carbocyclic or heterocyclic ring is optionally substituted with any one or more of the groups R₁ or R₂;

15

Z is selected from

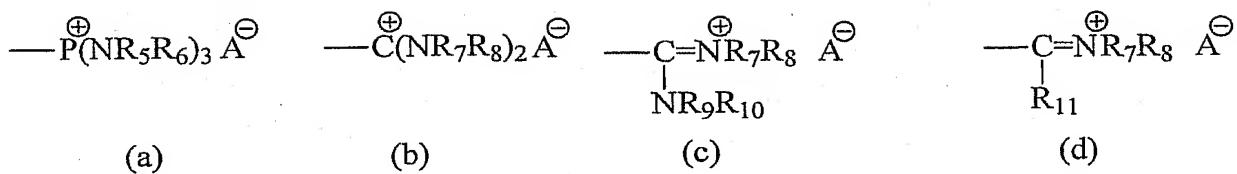
20 i) hydrogen;
 ii) P(O)(OR_{4a})₂ wherein R_{4a} is hydrogen, alkyl or aryl;
 iii) a N-protected amino acid represented by the structure



wherein R^α is an amino acid residue and Prt is a N-protecting group;

iv) C(O)R_{4b} wherein R_{4b} is alkyl or aryl; and
 v) a group represented by the formula (a), (b), (c), or (d):

25

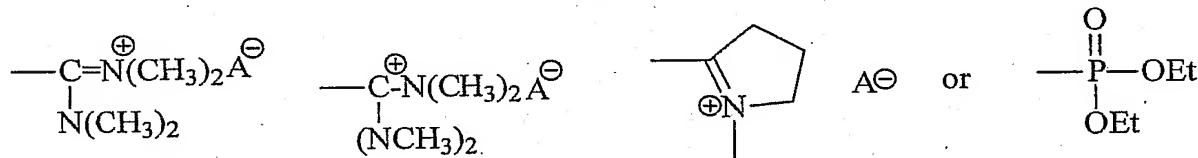


5 wherein R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} and R_{11} are independently of each other alkyl, aryl, or cycloalkyl; or one or more of (i) R_5 and R_6 , (ii) R_7 and R_8 , (iii) R_9 and R_{10} , (iv) R_7 and R_{11} , or (v) R_8 and R_{11} , together with the carbon or nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heterocyclic or heteroaryl ring which can optionally contain at least one further nitrogen, oxygen or sulphur in any ring part;
10 and

10

A is PF_6^- , BF_4^- , Br^- , Cl^- , SbF_6^- , SbCl_6^- , ClO_4^- , AlCl_4^- or any anion which forms a stable salt, soluble in organic solvents;

with the proviso that when Y is oxygen and Z is hydrogen or one of the groups:



15 R_1 and R_2 together with the carbon atoms to which they are attached are not an unsubstituted phenyl group;

and ammonium salts or N-oxides thereof.

2. The compound according to claim 1, wherein Y is O.

3. The compound according to claim 1, wherein Z is hydrogen.

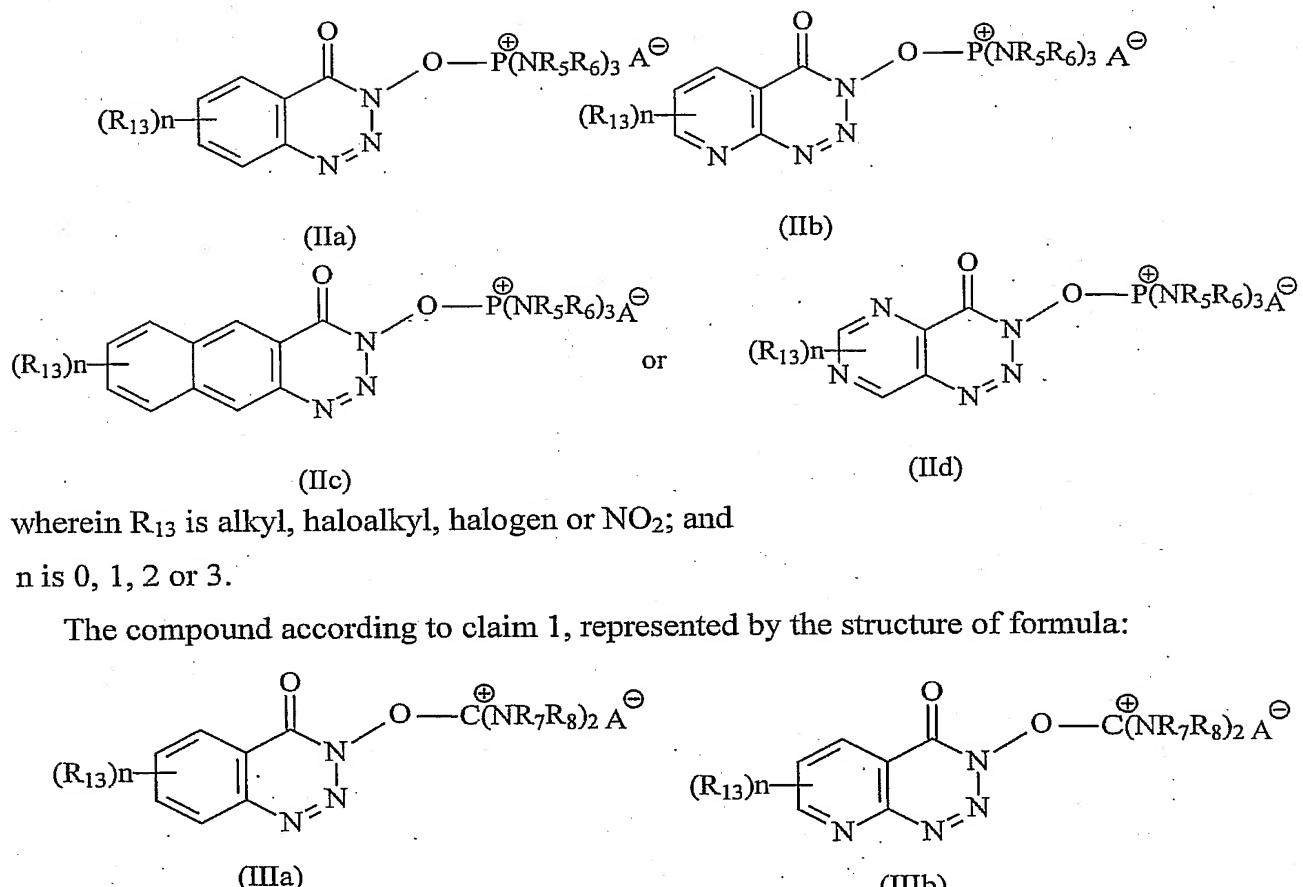
20 4. The compound according to claim 1, wherein R₁ and R₂ together with the carbon atoms to which they are attached form an optionally substituted phenyl ring.

5. The compound according to claim 1, wherein A is PF₆.

6. The compound according to claim 1, wherein A is BF₄.

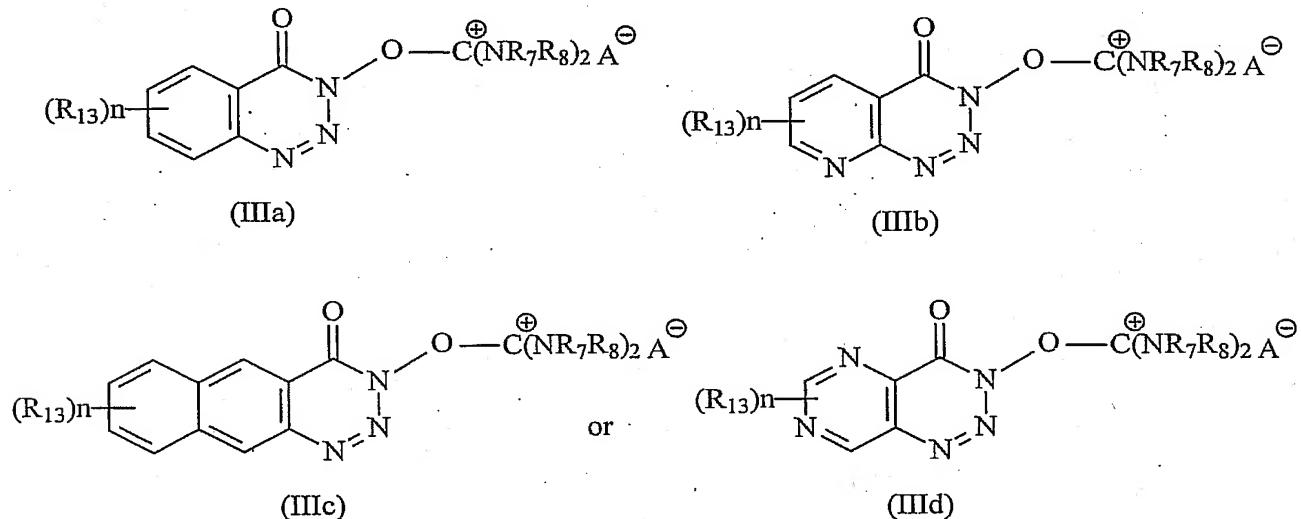
7. The compound according to claim 1, represented by the structure of formula:

25



wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and
 n is 0, 1, 2 or 3.

8. The compound according to claim 1, represented by the structure of formula:



5

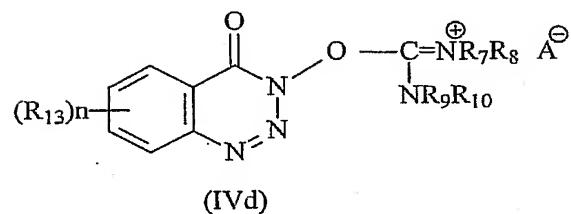
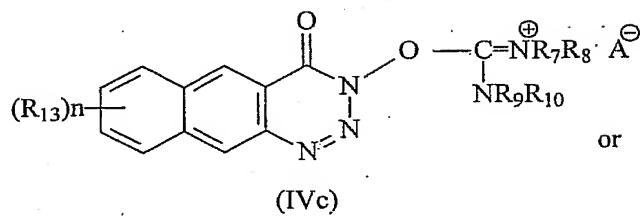
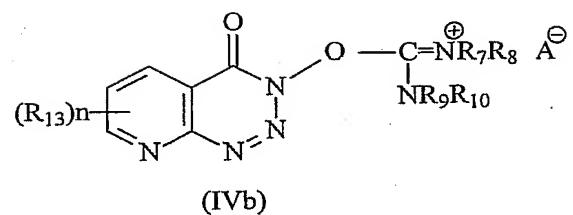
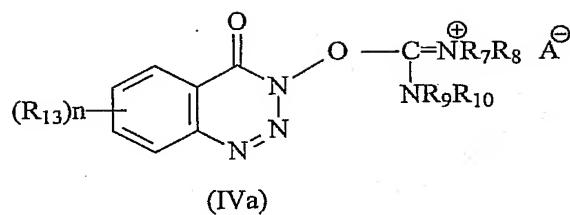
wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

n is 0, 1, 2 or 3;

with the proviso that for the compound of formula IIIa, when R_7 and R_8 are both methyl,

10 n is not 0.

9. The compound according to claim 1, represented by the structure of formula:



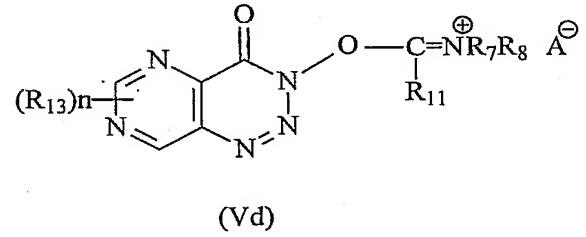
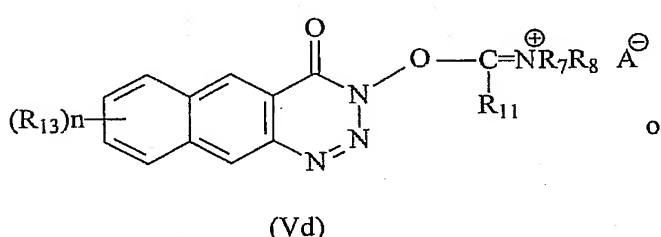
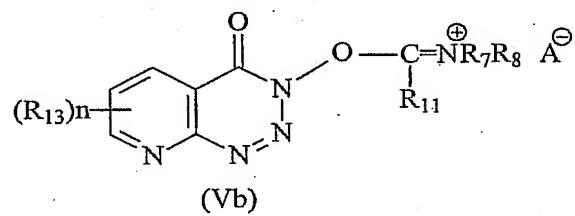
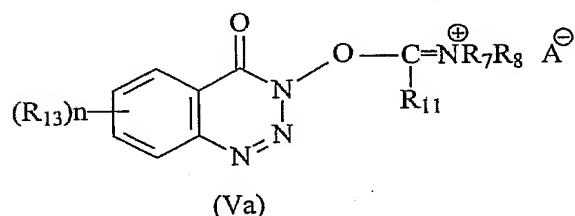
wherein R₁₃ alkyl, haloalkyl, halogen or NO₂; and

n is 0, 1, 2 or 3;

with the proviso that for the compound of formula IVa, when R_7 , R_8 , R_9 and R_{10} are all methyl, n is not 0.

5

10. The compound according to claim 1, represented by the structure of formula:

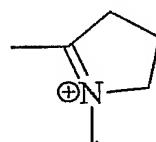


wherein R₁₃ alkyl, haloalkyl, halogen or NO₂; and

10

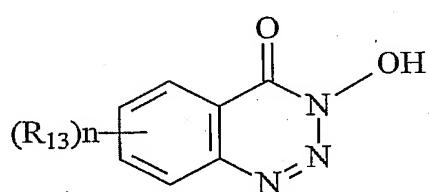
n is 0, 1, 2 or 3;

with the proviso that when R_7 , R_8 and R_{11} together with the carbon and nitrogen atoms to which they are attached represent a group of the formula:

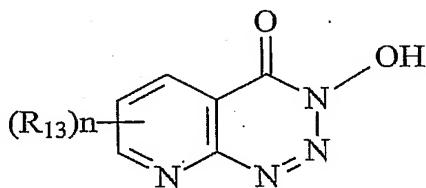


n is not 0.

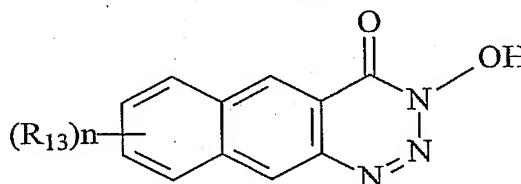
11. The compound according to claim 1, represented by the structure of formula:



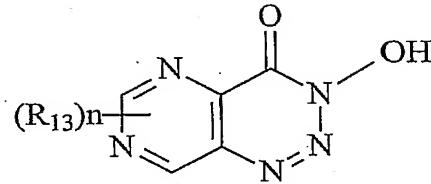
(VIa)



(VIIb)



or



(VIc)

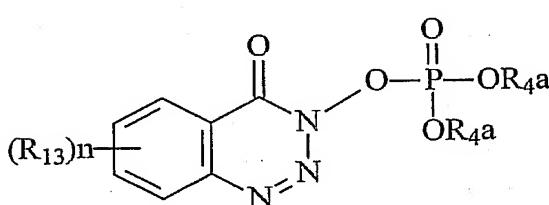
(VID)

5 wherein R_{13} alkyl, haloalkyl, halogen or NO_2 ; and

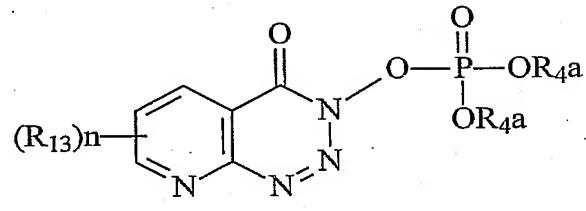
n is 0, 1, 2 or 3.

with the proviso that for the compound of formula VIa n is not 0.

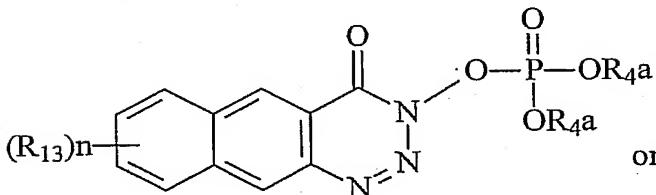
12. The compound according to claim 1, represented by the structure of formula



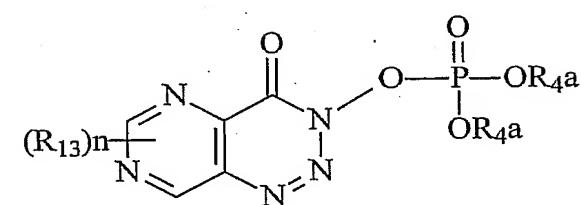
(VIIa)



(VIIb)



or



(VIIc)

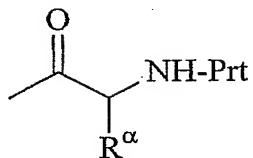
(VID)

10 wherein R_{13} is alkyl, haloalkyl, halogen or NO_2 ; and

n is 0, 1, 2 or 3;

with the proviso that for the compound of formula VIIa, when R_{4a} is ethyl, n is not 0.

13. The compound according to claim 1, wherein Z is a N-protected amino acid residue represented by the structure



5 14. The compound according to claim 13, wherein the N-protecting group is Fmoc, BOC, TEOC, Aoc, Adoc, Bpoc, Azoc, Ddz, Poc, Foc, Moz, Nps, Dts, Cbz, Bspoc, Bsmoc, Nps, formyl, acetyl or trifluoroacetyl.

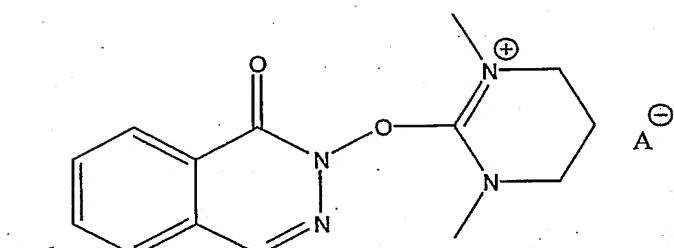
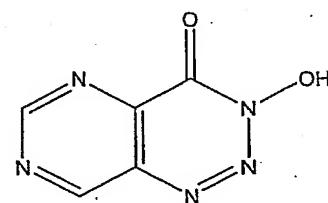
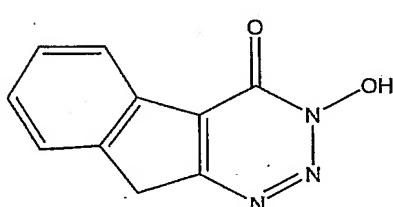
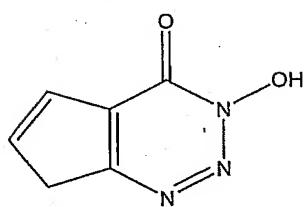
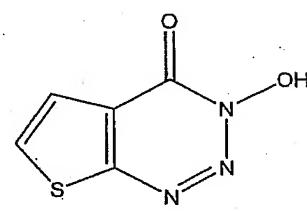
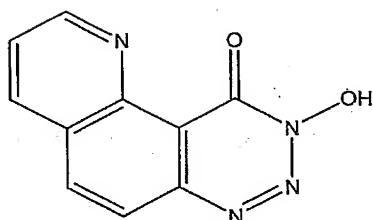
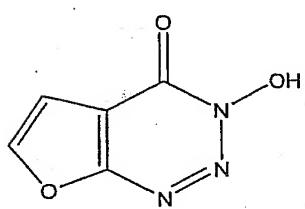
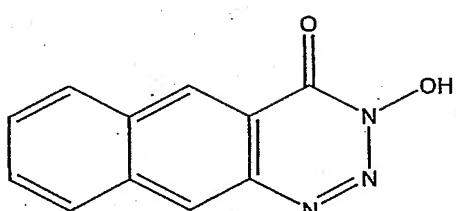
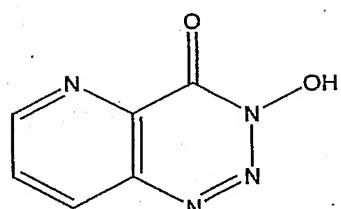
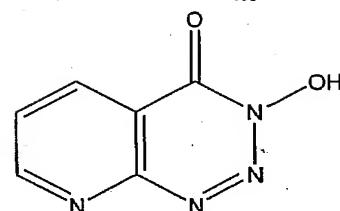
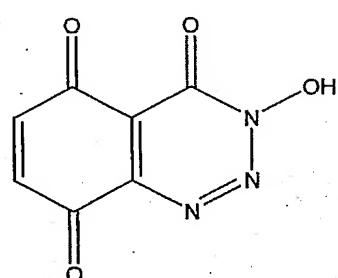
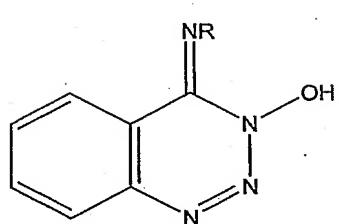
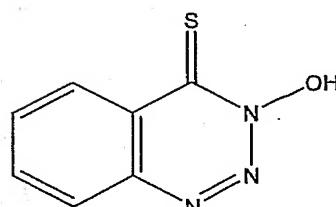
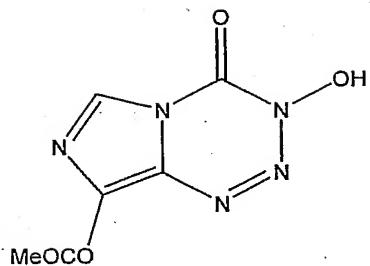
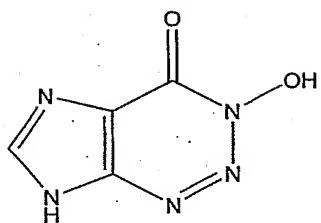
15. The compound according to claim 13, wherein the N-protected amino acid is selected from the group consisting of N- α -amino protected residue of glycine, alanine, valine, leucine, isoleucine, proline, arginine, lysine, histidine, serine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, cysteine, cystine, methionine, ornithine, norleucine, phenylalanine, tyrosine, tryptophan, beta-alanine, homoserine, homoarginine, isoglutamine, pyroglutamic acid, gamma-aminobutyric acid, citrulline, sarcosine, and statine.

10 16. The compound according to claim 13 wherein the N-protected amino acid is a side-group protected amino acid.

15 17. The compound according to claim 16 wherein the N-protected amino acid is selected from the group of amino acids consisting of arginine, lysine, aspartic acid, asparagine, glutamic acid, glutamine, histidine, cysteine, ornithine, serine, threonine, homoarginine, citrulline and tyrosine.

20 18. The compound according to claim 1, wherein R_1 and R_2 together with the carbon atoms to which they are attached form a ring selected from the group consisting of phenyl, naphthyl, thienyl, benzothienyl, 1-naphthothienyl, thianthrenyl, furyl, benzofuryl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, indazolyl, purinyl, isoquinolyl, quinolyl, naphthyridinyl, quinazolinyl, cinnolinyl, pteridinyl, carbolinyl, isothiazolyl, isoxazolyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclohexadienyl, and cycloheptadienyl.

25 19. The compound according to claim 1, wherein said compound is selected from the group consisting of:



and

20. A process for preparing a peptide bond comprising reacting an amino compound with a carboxylic acid in the presence of an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

5

21. A process for preparing a peptide bond comprising reacting an amino compound and a carboxylic acid in the presence of a dehydrating reagent and an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

10

22. The process according to claim 21, wherein said dehydrating reagent is EDC or DCC.

23. A process for preparing a peptide bond comprising reacting an amino compound and a carboxylic acid in the presence of a coupling reagent and an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

15

24. The process according to claim 23, wherein the coupling reagent is phosphonium, uronium or immonium-base coupling reagents.

25. The process according to claim 23, wherein the coupling reagent is BOP, PyBOP, HBTU or TBTU.

20

26. A process for preparing a peptide bond comprising reacting an amino compound and an acylating derivative of a carboxylic acid in the presence of an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

25

27. The process according to claim 26, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group.

28. A process for preparing a peptide bond comprising reacting an amino compound and an acylating derivative of a carboxylic acid in the presence of a dehydrating reagent and an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.

30

29. The process according to claim 29, wherein said dehydrating reagent is EDC or DCC.
30. The process according to claim 29, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group.
- 5 31. A process for preparing a peptide bond comprising reacting an amino compound and an acylating derivative of a carboxylic acid in the presence of a coupling reagent and an effective amount of a compound according to claim 1; wherein said amino compound is an amino acid or peptide and said carboxylic acid is an amino acid which is optionally N-protected or a peptide which is optionally N-terminal amino protected.
- 10 32. The process according to claim 31, wherein the coupling reagent is BOP, PyBOP, HBTU or TBTU.
33. The process according to claim 31, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group.
- 15 34. A process for the synthesis of a peptide, comprising the steps of:
 - a) providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;
 - b) optionally cleaving said N-protecting group to produce a free amino group;
 - c) coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of an effective amount of a compound according to claim 1;
 - 20 d) repeating steps (b) and (c) until the desired peptide has been obtained; and
 - e) cleaving the peptide from the resin.
35. A process for the synthesis of a peptide, comprising the steps of:
 - a) providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;
 - b) optionally cleaving said N-protecting group to produce a free amino group;
 - c) coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of a dehydrating reagent and an effective amount of a compound according to claim 1;
 - 30 d) repeating steps (b) and (c) until the desired peptide has been obtained; and
 - e) cleaving the peptide from the resin.
36. The process according to claim 35, wherein the dehydrating reagent is EDC or DCC.

37. A process for the synthesis of a peptide, comprising the steps of:

- providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;
- optionally cleaving said N-protecting group to produce a free amino group;
- coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of a coupling reagent and an effective amount of a compound according to claim 1;
- repeating steps (b) and (c) until the desired peptide has been obtained; and
- cleaving the peptide from the resin.

10 38. The process according to claim 37, wherein said coupling reagent is BOP, PyBOP, HBTU or TBTU.

39. A process for the synthesis of a peptide, comprising the steps of:

- providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;
- optionally cleaving said N-protecting group to produce a free amino group;
- coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of an effective amount of a compound according to claim 1;
- repeating steps (b) and (c) until the desired peptide has been obtained; and
- cleaving the peptide from the resin.

15 40. The process according to claim 39, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group.

20 41. A process for the synthesis of a peptide, comprising the steps of:

- providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;
- optionally cleaving said N-protecting group to produce a free amino group;
- coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of a dehydrating reagent an effective amount of a compound according to claim 1;
- repeating steps (b) and (c) until the desired peptide has been obtained; and
- cleaving the peptide from the resin.

42. The process according to claim 41, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group.

43. The process according to claim 41, wherein the dehydrating reagent is EDC or DCC.

5 44. A process for the synthesis of a peptide, comprising the steps of:

10 a) providing a first amino acid which is optionally N-protected, wherein said amino acid is covalently coupled to a solid phase peptide synthesis resin;

b) optionally cleaving said N-protecting group to produce a free amino group;

c) coupling the free amino group via a peptide linkage to the carboxyl group of a second amino acid which is optionally N-protected, in the presence of a coupling reagent and an effective amount of a compound according to claim 1;

d) repeating steps (b) and (c) until the desired peptide has been obtained; and

e) cleaving the peptide from the resin.

45. The process according to claim 44, wherein the acylating derivative is an acyl chloride, anhydride or a phenoxy ester wherein the phenyl group is substituted with electron withdrawing group

15 46. The process according to claim 44, wherein said coupling reagent is BOP, PyBOP, HBTU or TBTU.

47. The process according to any of claims 30-46 wherein the N-protecting group is Fmoc, Boc, Teoc, Aoc, Adoc, Bpoc, Azoc, Ddz, Poc, Foc, Moz, Nps, Dts, Cbz, Bspoc, Bsmoc, Nps, formyl, acetyl or trifluoroacetyl.

20 48. The process according to any of claims 30-46, wherein the N-protected amino acid is naturally occurring amino acid.

49. The process according to any of claims 30-46, wherein the N-protected amino acid is selected from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, arginine, lysine, histidine, serine, threonine, aspartic acid, glutamic acid, asparagine, glutamine, cysteine, cystine, methionine, ornithine, norleucine, phenylalanine, tyrosine, tryptophan, beta-alanine, homoserine, homoarginine, isoglutamine, pyroglutamic acid, gamma-aminobutyric acid, citrulline, sarcosine, and statine.

25 50. The process according to claims 30-49, wherein the polypeptide chain is synthesized on an insoluble solid support and wherein said compound of formula I is coupled to said insoluble support.

51. The process according to claim 50, wherein said insoluble support is a phenyl ester-type resin.
52. The process according to claim 50, wherein said resin is one with oxime or thioester linkages.
- 5 53. A process for forming an amide bond comprising reacting an organic amine and a carboxylic acid in the presence of an effective amount of the compound according to claim 1.
54. A process for forming an amide bond comprising reacting an organic amine with a carboxylic acid in the presence of a dehydrating reagent and an effective amount of the compound according to claim 1.
- 10 55. The process according to claim 54, wherein said dehydrating reagent is EDC or DCC.
56. A process for forming an amide bond comprising reacting an organic amine with a carboxylic acid in the presence of a coupling reagent and an effective amount of the compound according to claim 1.
57. The process according to claim 56, wherein said coupling reagent is BOP, PyBOP, HBTU or TBTU.
- 15 58. A process for forming an amide bond comprising reacting an organic amine with an acylating derivative of a carboxylic acid in the presence of an effective amount of the compound according to claim 1.
59. The process according to claim 58, wherein the acylating derivative is an acyl chloride, anhydride, an alkyl ester or a phenoxy ester wherein the alkyl or phenyl group is substituted with at least one electron withdrawing group.
- 20 60. A process for forming an amide bond comprising reacting an organic amine with an acylating derivative of a carboxylic acid in the presence of a dehydrating agent an effective amount of the compound according to claim 1.
- 25 61. The process according to claim 60, wherein the acylating derivative is an acyl chloride, anhydride, an alkyl ester or a phenoxy ester wherein the alkyl or phenyl group is substituted with at least one electron withdrawing group.
62. The process according to claim 60, wherein said dehydrating reagent is EDC or DCC.
- 30 63. A process for forming an amide bond comprising reacting an organic amine with an acylating derivative of a carboxylic acid in the presence of a coupling reagent and an effective amount of the compound according to claim 1.

64. The process according to claim 63, wherein the acylating derivative is an acyl chloride, anhydride, an alkyl ester or a phenoxy ester wherein the alkyl or phenyl group is substituted with at least one electron withdrawing group.

65. The process according to claim 63, wherein said coupling reagent is BOP, PyBOP, HBTU or TBTU.

66. A process for forming an ester bond comprising reacting an organic alcohol and a carboxylic acid in the presence of an effective amount of the compound according to claim 1.

67. A process for forming an ester bond comprising reacting an organic alcohol with an acylating derivative of a carboxylic acid in the presence of an effective amount of the compound according to claim 1.

68. The process according to claim 67, wherein the acylating derivative is an acyl chloride, anhydride, an alkyl ester or a phenoxy ester wherein the alkyl or phenyl group is substituted with at least one electron withdrawing group.

69. A compound which is selected from:

15 7-Chloro-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine;
3,4-Dihydro-3-hydroxy-7-methyl-4-oxo-1,2,3-benzotriazine;
3,4-Dihydro-3-hydroxy-6,7-dimethyl-4-oxo-1,2,3-benzotriazine;
3,4-Dihydro-3-hydroxy-8-trifluoromethyl-4-oxo-1,2,3-benzotriazine;
6-Chloro-3,4-Dihydro-3-hydroxy-7-nitro-4-oxo-1,2,3-benzotriazine;

20 3,4-Dihydro-3-hydroxy-6-fluoro-4-oxo-1,2,3-benzotriazine;
3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,8-tetraazanaphthalene;
3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-triazaanthracene;
3,4-Dihydro-3-hydroxy-4-oxo-1,2,3,4,8-pentaazanaphthalene;
3-(Diethoxyphosphoryloxy)-1,2,3,8-tetraazanaphthalene -4(3H)-one;

25 7-Chloro-3-(diethoxyphosphoryloxy)-1,2,3-benzotriazine -4(3H)-one;
O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate;

30 O-(3,4-Dihydro-4-oxo-1,2,3,4,8-pentaazanaphthalen-3-yl)-N,N,N',N'-O-(3,4-Dihydro-6-fluoro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate;
O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-bis(tetramethylene)uronium hexafluorophosphate;
O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-bis(pentamethylene)uronium hexafluorophosphate;

O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'-
O-(5-Chloro-3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'-
trimethyleneuronium hexafluorophosphate;

5 O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N,N',N'-tetramethyluronium
tetrafluoroborate.

3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-N,N'-1,3-dimethyl-N,N'-dimethyleneuronium
tetrafluoroborate;

[(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-tris(dimethylamino)- phosphonium
hexafluorophosphate;

10 [(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-tris(pyrrolidino)- phosphonium
hexafluorophosphate; and

2-[(3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]-1,1-dimethyl-2-pyrrolidin-1-yl-1,3,2-
diazaphospholidinium hexafluorophosphate.

15

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,075,141 A (CAREY) 13 June 2000 (13.06.2000), see entire document, especially column 4.	1-4 and 13-69
X	US 5,952,497 A (CAREY) 14 September 1999 (14.09.1999), see entire document especially column 4.	1-4 and 13-69
X	WADA et al., Chemical Synthesis of Oligodeoxyribonucleotides using N-unprotected H-Phosphonate Monomers and Carbonium and Phosphonium Condensing Reagents: O-Selective Phosphonylation and Condensation. J. Am. Chem. Soc. 119, 12710-12721, 1997.	1-12
X	CARPINO et al., Efficiency in Peptide Coupling: 1-Hydroxy-7-azabenzotriazole vs 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine. J. Org. Chem. 60, 3561-3564, 1995.	1-69

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"O" document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
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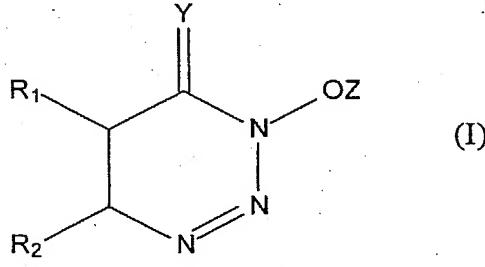
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(54) Title: 3-HYDROXY-4-OXO-1,2,3-TRIAZINES AND DERIVATIVES THEREOF FOR AMIDE AND ESTER BOND FORMATION



(57) Abstract: The present invention relates to the use of a compound of formula I as a coupling reagent in forming amide or ester bonds from a reaction between a carboxylic acid and an amine or an alcohol, respectively. The compounds of formula I are especially useful as coupling reagents in the preparation of peptide bonds during peptide synthesis. In particular, the compounds of formula I are useful in promoting the formation of reactive reaction intermediates, inhibiting side reactions and in suppression of racemization. In addition, the present invention provides novel compounds of Formula I, and salts of N-oxides thereof.

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